

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
15 August 2002 (15.08.2002)

PCT

(10) International Publication Number  
**WO 02/062369 A2**

(51) International Patent Classification<sup>7</sup>: **A61K 38/00**

(21) International Application Number: PCT/US02/03201

(22) International Filing Date: 4 February 2002 (04.02.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/265,955 2 February 2001 (02.02.2001) US

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: **UROGUANYLIN AND CYCLOOXYGENASE-2 INHIBITOR COMBINATIONS FOR INHIBITION OF INTESTINAL CANCER**

(57) Abstract: Disclosed is a method of retarding the development of polyps and prevention, inhibition and treatment of cancer in the intestine of a subject by administration of a composition comprising a peptide with the active domain of uroguanylin or any agonist peptide or compound binding to the guanylate cyclase receptor GC-C in the intestine in combination with a naturally occurring, derived from a naturally occurring, or a chemically synthesized cyclooxygenase-2 inhibitor, preferably a selective cyclooxygenase-2 inhibitor.

WO 02/062369 A2

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UROGUANYLIN AND CYCLOOXYGENASE-2 INHIBITOR COMBINATIONS  
FOR INHIBITION OF INTESTINAL CANCER

BACKGROUND OF THE INVENTION

5       The present invention relates to the use of certain peptides, more particularly the use of uroguanylin and prouroguanylin in combination with any one of or combination of naturally occurring, extract of a naturally occurring, or a chemically synthesized  
10 cyclooxygenase-2 inhibitor, preferably a selective cyclooxygenase-2 inhibitor or inhibitors, to retard the development of polyps and prevent, inhibit or treat cancer in the intestine.

      The pathogenesis of colorectal cancer is  
15 characterized as a multistep process that begins with increased proliferation and/or decreased apoptosis of colorectal epithelial cells resulting in generation of polyps, followed by adenoma formation and ultimately to adenocarcinoma. Certain individuals develop multiple  
20 colorectal adenomas and subsequent carcinomas early in life because of a genetic defect in the APC gene responsible for causing a condition called familial adenomatous polyposis (FAP). Dihlmann et al, *Dominant negative effect of the APC 1309 mutation: a possible explanation for genotype-phenotype correlations in familial adenomatous polyposis*, Cancer Res. 1999 Apr. 15, 59(8): 1857-60. Chemoprevention has evolved during the last decade as a viable strategy for cancer prevention, with the aim of controlling the development  
25 of cancer through pharmacological and/or dietary intervention prior to the appearance of a clinically detectable tumor. Reddy, B.S. (1997) *Chemoprevention of colon cancer by dietary administration of naturally-occurring and related synthetic agents*, Adv. Exp. Med. Biol. 400B:931-936.  
30  
35

Uroguanylin and guanylin are structurally related enteric peptide hormones that are secreted intraluminally by different types of cells, include enterochromaffin, goblet and others within the intestinal mucosal lining. A receptor for these peptides that has been identified at the molecular level is a transmembrane form of guanylate cyclase (GC) known as GC-C. Krause, W.J. et al, *The guanylin and uroguanylin peptide hormones and their receptors*, Acta. Anat. (Basel) 160:213-231 (1997). GC-C receptors are localized on the luminal surface of enterocytes throughout the GI tract. Swenson, E.S. et al, *The guanylin/STa receptor is expressed in crypts and apical epithelium throughout the mouse intestine*, Biochem. Biophys. Res. Commun. 225:1009-1014 (1996). Binding of uroguanylin or guanylin to the extracellular domain of GC-C receptors stimulates intracellular production of the second messenger cGMP, resulting in activation of cystic fibrosis transmembrane conductance regulator (CFTR), the apical membrane channel for efflux of chloride from enterocytes lining the intestinal tract. Forte, L.R. et al, *Salt and water homeostasis: uroguanylin is a circulating peptide hormone with natriuretic activity*, Am. J. Kidney Dis. 28:296-304 (1996). Activation of CFTR chloride channel proteins and the subsequent enhancement of transepithelial secretion of chloride leads to stimulation of sodium ( $\text{Na}^+$ ) and water secretion into the intestinal lumen. Forte, L.R. et al, *Guanylin regulatory peptides: structures, biological activities mediated by cyclic GMP and pathobiology*, Regul. Pept. 81:25-39 (1999). Therefore, one of the major physiological functions of these hormones is the regulation of fluid and electrolyte transport in the gastrointestinal (GI) tract by serving as paracrine regulators of CFTR activity.

The precursor of uroguanylin is prouroguanylin, which is broken down by endogenous proteases in the intestinal tract to produce the active uroguanylin. Chymotrypsin activates prouroguanylin to cleave it into  
5 its active form of uroguanylin. Forte, et al, *Salt and Water Homeostasis: Uroguanylin Is a Circulating Peptide Hormone With Natriuretic Activity*, Am. J. Kid. Dis. 1996, 28, No.2, 296-304. Uroguanylin is an acid-stable and proteolysis-resistant peptide, which will remain in-  
10 tact to act on the intestinal lumen directly rather than being absorbed systemically. Uroguanylin and guanylin are produced throughout the intestinal mucosa and in the myocardium. Forte et al, *Salt and water homeostasis: uroguanylin is a circulating peptide hormone with natriuretic activity* Am. J. Kidney Dis. 28:296-304  
15 (1996). Human uroguanylin has been isolated from human urine and has been chemically synthesized by solid phase peptide synthesis as described in U.S. Patent Number 5,489,670 for *Human Uroguanylin*.

20 Binding of uroguanylin or guanylin to the guanylin cyclase receptor stimulates the intracellular production of the cGMP ultimately resulting in the stimulation of salt and water secretion into the intestinal lumen. Uroguanylin and guanylin receptors are found on the  
25 luminal surface of epithelial cells lining the intestinal tract and renal proximal tubules as well as in other organs. Forte et al, *Salt and Water Homeostasis: Uroguanylin Is a Circulating Peptide Hormone with Natriuretic Activity*, Am. J. Kid. Dis. 1996,  
30 28, No. 2, 296-304. Uroguanylin has been found to stimulate increases in cyclic GMP levels in a manner similar to another family of heat stable enterotoxins (STs) secreted by pathogenic strains of E. coli and other enteric bacteria that activate intestinal  
35 guanylate cyclase and cause secretory diarrhea, which is

a major cause of traveler's diarrhea and many deaths in developing countries. Forte et al, *Lymphoguanylin: Cloning and Characterization of a Unique Member of the Guanylin Peptide Family*, Endocrinology Vol. 140, No. 4, p.1800-1806. These ST peptides act as molecular mimics of the endogenous mammalian peptides of uroguanylin and prouroguanylin. Forte et al, Endocrinology Vol. 140, No. 4, p.1800. Unlike uroguanylin the STs from enteric bacteria do not have a decrease in potency when the pH changes in the colon. STs are more potent than either uroguanylin or guanylin under both acidic and alkaline conditions. Forte et al, *Guanylin: a peptide regulator of epithelial transport*, The FASEB Journal, vol. 9, 643-650 (1995). Uroguanylin is believed to regulate fluid and electrolyte transport in a manner similar to guanylin and the STs in the GI tract. Therefore, as mentioned in previous publications the human uroguanylin may act as a laxative and be useful in patient suffering from constipation.

Prostaglandins play a major role in the inflammation process and the inhibition of prostaglandin production, especially production of PGG<sub>2</sub>, PGH<sub>2</sub> and PGE<sub>2</sub>, has been a common target of anti-inflammatory drug discovery. However, common non-steroidal anti-inflammatory drugs (NSAID's) that are active in reducing the prostaglandin-induced pain and swelling associated with the inflammation process are also active in affecting other prostaglandin-regulated processes not associated with the inflammation process. Thus, use of high doses of most common NSAID's can produce severe side effects, including life threatening ulcers, that limit their therapeutic potential. An alternative to NSAID's is the use of corticosteroids, which also produce severe adverse effects, especially when long term therapy is involved.

NSAIDs have been found to prevent the production of prostaglandins by inhibiting enzymes in the human arachidonic acid/prostaglandin pathway, including the enzyme cyclooxygenase (COX). The recent discovery of an  
5 inducible enzyme associated with inflammation (named "cyclooxygenase-2 (COX-2)" or "prostaglandin G/H synthase II") provides a viable target of inhibition which more effectively reduces inflammation and produces fewer and less drastic side effects.

10 Compounds that selectively inhibit cyclooxygenase-2 have also been described in the following individual publications.

U.S. Patent No. 5,380,738.  
U.S. Patent No. 5,344,991.  
15 U.S. Patent No. 5,393,790.  
U.S. Patent No. 5,434,178.  
U.S. Patent No. 5,474,995.  
U.S. Patent No. 5,510,368.  
WO 96/06840.  
20 WO 96/03388.  
WO 96/03387.  
WO 96/19469.  
WO 96/25405.  
WO 95/15316.  
25 WO 94/15932.  
WO 94/27980.  
WO 95/00501.  
WO 94/13635.  
WO 94/20480.  
30 WO 94/26731.

Further, natural Cyclooxygenase-2 inhibitors have been disclosed in "Selective Cyclooxygenase-2 Inhibition

from Edible Plant Extracts", US Non-provisional Application number 09/737892, filed Jan. 03, 2001; "Selective Cyclooxygenase-2 Inhibition from Non-edible Plant Extracts", US Non-provisional Application number  
5 09/737701, filed Jan. 03, 2001; and "Selective Cyclooxygenase-2 Inhibition from Plant Extracts", US Non-provisional Application number 09/738041, filed Jan. 03, 2001. [Pyrazol-1-yl]benzenesulfonamides have been described as inhibitors of cyclooxygenase-2 and have  
10 shown promise in the treatment of inflammation, arthritis, and pain, with minimal side effects in pre-clinical and clinical trials. Their use for preventing colon cancer has been described in U.S. Patent No. 5,466,823. However, their use for treating or preventing  
15 intestinal cancer, in combination with uraguanilin, has not been described.

#### SUMMARY OF THE INVENTION

20

The combination of certain peptides, particularly uroguanylin or prouroguanylin, with a single or multiple natural occurring, extract of a natural occurring, or a chemically synthesized cyclooxygenase-2 inhibitor,  
25 preferably a selective cyclooxygenase-2 inhibitor or inhibitors, may be useful in treating, preventing, inhibiting or retarding the development of polyps and cancer in the intestine.

Among the objects and features of the present  
30 invention may be noted the provision of a process for retarding the development of polyps and preventing, and a process for inhibiting and treating cancer or neoplasia in a subject. Preferably the method is useful for treating the development of polyps and preventing,  
35 and a process for inhibiting and treating cancer in the

intestine of a subject, more preferably the small intestine or the colon.

Briefly, therefore, the present invention is directed to a process for retarding the development of polyps in a subject which comprises the administration  
5 of a peptide including the amino acid sequence:

Asp- Asp- Cys- X<sub>1</sub>- X<sub>2</sub>- Cys- X<sub>3</sub>- Asn- X<sub>4</sub>- X<sub>5</sub>- Cys- X<sub>6</sub>- X<sub>7</sub>-  
Cys

10

wherein each of X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub>, X<sub>5</sub>, X<sub>6</sub>, and X<sub>7</sub> is an amino acid residue, and the polypeptide is cross-linked by a disulfide bond between the cystine residue immediately adjacent the amine group of X<sub>1</sub> and the cystine residue  
15 immediately adjacent the amine group of X<sub>6</sub> and by a disulfide bond between the cystine residue immediately adjacent the amine group of X<sub>3</sub> and the cystine residue immediately adjacent the carboxy group of X<sub>7</sub>, in combination with any one of or combination of naturally  
20 occurring, or an extract of a natural occurring, or a chemically synthesized cyclooxygenase-2 inhibitor, preferably a selective cyclooxygenase-2 inhibitor or inhibitors.

US Patent application number PCT/US00/21998 (herein  
25 incorporated by reference) describes the use of uroguanylin as an intestinal cancer inhibiting agent.



The invention is further directed to a process for retarding the development of polyps and to a process for the prevention, inhibition or treatment of cancer in a subject by administration of a composition comprising  
5 any one of or combination of the following peptides: uroguanylin, human uroguanylin, pro-uroguanylin, and human pro-uroguanylin, guanylin, lymphoguanylin, prolymphoguanylin and heat stable enterotoxin in combination with any one of or combination of naturally  
10 occurring, or an extract of a natural occurring, or a chemically synthesized cyclooxygenase-2 inhibitor, preferably a selective cyclooxygenase-2 inhibitor or inhibitors.

Additionally, the invention is directed to a  
15 process for retarding the development of polyps and a process for the prevention, inhibition or treatment of cancer by administration of a composition comprising any one of or a combination of agonist peptides and/or other agonist compounds to the guanylate cyclase receptor GC-C  
20 in combination with any one of or combination of naturally occurring, or an extract of a natural occurring, or a chemically synthesized cyclooxygenase-2 inhibitor, preferably a selective cyclooxygenase-2 inhibitor or inhibitors.

25 The cancer or neoplasia which can be treated with the present inventive method can be located anywhere in the body, for example, the head, neck, chest, lungs, skin, liver, blood, kidneys, heart, intestines, bladder, gall bladder, brain, throat, musculoskeletal system,  
30 lymphatic system, central nervous system, and others. Preferably, the methods of the present invention are used to treat cancer or neoplasia located in the intestine, for example, the small intestine or colon.

Other objects of this invention will be in part apparent and, in part, pointed out hereinafter.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

5

The term "treatment" includes partial or total inhibition of the tumor growth, either benign or malignant, spreading or metastasis, as well as partial or total destruction of the neoplastic cells.

10

The term "prevention" includes either preventing the onset of clinically evident neoplasia altogether or preventing the onset of a preclinically evident stage of neoplasia in individuals at risk. Also intended to be encompassed by this definition is the prevention of  
15 initiation for malignant cells or to arrest or reverse the progression of premalignant cells to malignant cells. This includes prophylactic treatment of those at risk of developing the neoplasia.

The phrase "therapeutically-effective" is intended  
20 to qualify the amount of each agent which will achieve the goal of improvement in disease severity and the frequency of incidence over treatment of each agent by itself, while avoiding adverse side effects typically associated with alternative therapies.

25

The term "subject" for purposes of treatment includes any human or animal subject who has any one of the known neoplasia or tumor disorders, and preferably is a human subject. For methods of prevention, the subject is any human or animal subject, and preferably  
30 is a human subject who is at risk for obtaining an intestinal cancer or neoplasia-related disorder, either

benign or malignant, including metastasis. The subject may be at risk due to exposure to carcinogenic agents, being genetically predisposed to have the neoplasia, and the like.

5       The term neoplasia includes both benign and cancerous tumors and growths.

          In the method above, the epithelial cell-derived neoplasia includes epithelial carcinomas such as basal cell carcinoma, adenocarcinoma, colon cancer, prostate  
10 cancer, renal cell carcinoma, and other known neoplasias that effect epithelial cells throughout the body. Preferably, the epithelial cell-derived neoplasia is selected from gastrointestinal cancer, liver cancer, prostate cancer, kidney cancer, brain cancer, bladder  
15 cancer, cervical cancer, lung cancer, breast cancer and skin cancer.

          Inhibitors of the cyclooxygenase pathway in the metabolism of arachidonic acid used in the prevention and treatment of cancer or neoplasia  
20 may inhibit enzyme activity through a variety of mechanisms. The use of cyclooxygenase-2 selective inhibitors is highly advantageous in that it minimize the gastric side effects that can occur with non-selective NSAID's, especially  
25 where prolonged prophylactic treatment is expected.

          The term "cyclooxygenase-2 inhibitor" denotes a compound able to inhibit cyclooxygenase-2 without significant inhibition of cyclooxygenase-1. Preferably,  
30 it includes compounds which have a cyclooxygenase-2 IC50

of less than about 0.2  $\mu\text{M}$ , and also have a selectivity ratio of cyclooxygenase-2 inhibition over cyclooxygenase-1 inhibition of at least 50, and more preferably of at least 100. Even more preferably, the  
5 compounds have a cyclooxygenase-1  $\text{IC}_{50}$  of greater than about 1  $\mu\text{M}$ , and more preferably of greater than 10  $\mu\text{M}$ .

The term "purified" means partially purified and/or completely purified. Thus a "purified composition" may be either partially purified or completely purified. An  
10 extract of a naturally occurring cyclooxygenase-2 inhibitor may be partially purified or purified.

Uroguanylin is secreted naturally by the goblet cells of the intestinal mucosal lining as prouroguanylin, a functionally inactive form, which is  
15 then converted to the functionally active uroguanylin in the intestine by endogenous proteases. Uroguanylin is an acid-stable, proteolysis-resistant peptide. Therefore, orally delivered prouroguanylin and uroguanylin will act on the lumenal intestinal surface  
20 and not be absorbed systemically. Oral administration of uroguanylin, prouroguanylin and other like peptides, containing the amino acid sequences similar to the active domain, are expected to induce apoptosis, cell death, in the intestinal mucosal cell lining. The  
25 induced apoptosis in the intestinal mucosal cell lining is expected to retard the incidence of polyp formation and subsequent intestinal cancer. Without intending to be bound by any theory, applicants believe that the peptides of the invention exert their effects by  
30 increasing the rate of apoptosis, cell death, in the intestinal mucosal cell lining promoting the perfect balance between the cell proliferation and the programmed cell death thereby retarding the growth of polyps and preventing, inhibiting, and treating cancer

in the intestine and other epithelial-derived cancer possessing receptors for guanylin, uroguanylin, lymphoguanylin and STa family of peptides.

5 The rate of cell proliferation and cell death in the intestinal mucosa is very rapid. The cells of the intestinal mucosa are in a steady state of turnover to insure a perfect balance between cell proliferation and cell death. The constant rapid renewal of the GI tract epithelium fulfills the functions of maintaining the  
10 integrity of normal mucosa, repairing and replenishing differentiated epithelial cells that have specialized functions. The prevention of apoptosis in the intestinal mucosal cells creating an imbalance in the renewal process results in an increased incidence of polyp  
15 formation and subsequent intestinal cancer. See Eastwood et al, *A review of gastrointestinal epithelial renewal and its relevance to the development of adenocarcinomas of the gastrointestinal tract*, J. Clin. Gastroenterol. 21: S1-11 (1995). The process of  
20 apoptosis is known to be suppressed in colon cancer tissues. Baretton, et al, *Apoptosis and immunohistochemical bcl-2 expression in colorectal adenomas and carcinomas. Aspects of Carcinogenesis and prognostic significance*, Cancer 77:255-264 (1996).

25 A major cellular characteristic of the apoptotic process is a marked loss of cell volume, which is directly related to the movement of ions, with homeostasis being achieved by the balance of osmotic pressure across the plasma membrane. Hoffman, E.K. et  
30 al, *Membrane mechanisms in intracellular signalling in cell volume regulation*, Int. Rev. Cytol. 161:173-262 (1995). Most mammalian cells achieve and maintain this osmotic pressure through the continuous action of  $\text{Na}^+/\text{K}^+$  ATPase pump, which creates a gradient of these

monovalent cations across the membrane. Several sources of evidence have implicated a potential role of  $K^+$  efflux in the induction of apoptosis. Hughes, F.M. et al, *Intracellular  $K^+$  suppresses the activation of apoptosis in lymphocytes*, J.Biol.Chem. 272:30567-30576 (1997); Hughes, F.M. et al, *Potassium is a critical regulator of apoptotic enzymes in vitro and in vivo*, Adv. Enzyme Regul. 39:157-171 (1999). First, a bacterial pore-forming cytolysin, staphylococcal  $\alpha$ -toxin, which selectively permeabilizes plasma membranes for monovalent cations, was found to induce apoptosis. Bhakdi, S. et al, *Release of interleukin-1 beta associated with potent cytotoxic action of staphylococcal alpha-toxin on human monocytes*, Infect. Immun. 57:3512-3519 (1989). Second, apoptotic and shrunken cells have been shown to contain much lower levels of intracellular  $K^+$  as compared to that in normal cells. Hughes, F.M et al, *Intracellular  $K^+$  suppresses the activation of apoptosis in lymphocytes*, J.Biol.Chem. 272:30567-30576 (1997). Third, an intracellular  $K^+$  concentration more than 150mM has been shown to selectively inhibit Caspase-3, a proteolytic enzyme involved in the induction of apoptosis. Hughes, F.M. et al, *Potassium is a critical regulator of apoptotic enzymes in vitro and in vivo*, Adv.Enzyme Regul. 39:157-171 (1999). Finally, suppressing  $K^+$  efflux in whole cells prevents the activation of pro-apoptosis nucleases, whereas enhancing the efflux of this ion facilitates enzymatic activities of these nucleases. Hughes, F.M. 39: 157-171 (1999). Thus, intracellular levels of potassium balance appear to be the critical regulator of apoptosis.

Additionally, guanylin has been shown to be completely diminished in colon cancer cells and even

expressed in normal intestinal mucosal cells. This finding suggest that guanylin is involved in the maintenance of colonic differentiation or functions as a tumor modifier gene. Mitchell et al., *Guanylin mRNA Expression in Human Intestine and Colorectal Adenocarcinoma*, Lab. Invest. 1998, Vol. 78, No. 1, 101-108. Recent data demonstrates that the guanylin cyclase receptor known as GC-C receptor is expressed in all primary and metastatic colorectal cancers and it may serve as a specific marker for these tumors. Carrithers, S.L. et al, *Guanylin cyclase C is a selective marker for metastatic colorectal tumors in human extraintestinal tissues*, Proc. Natl. Acad. Sci. USA. 93:14827-14832. By contrast, the expression of guanylin has been shown to be down-regulated in colorectal cancer tissues and cell lines. Cohen, M.B. et al, *Guanylin mRNA expression in human intestine and colorectal adenocarcinoma*, Lab. Invest. 78:101-108.

In PCT/US00/21998 (herein incorporated by reference) uroguanylin was shown to be completely diminished in colon cancer cells and evenly distributed in normal intestinal mucosal cells. Additionally, the expression of uroguanylin and guanylin in human colon cancer and the adjacent normal tissues was reportedly completely diminished in all human colon cancer specimens examined. That study suggested that either the reduced expression of uroguanylin and/or guanylin leads to or is a result of adenocarcinoma formation. In the same application, it was demonstrated that treatment with uroguanylin resulted in the induction of apoptosis in T-84, human colon carcinoma cells, and that the oral administration of human uroguanylin leads to inhibition in polyp formation in the intestinal tract of Min-mouse,

an animal model for human Familial Adenomatous Polyposis (FAP).

Both guanylin and uroguanylin genes have recently been mapped on the mouse chromosome 4 and to a synthetic position on human chromosome 1p34-35. Sciaky, D. et al, Mapping of guanylin to murine chromosome 4 and human chromosome 1p34-35, Genomics 26:427-429 (1995); Whitaker, T.L. et al, The uroguanylin gene (*Guca 1b*) is linked to guanylin (*Guca 2*) on mouse chromosome 4, Genomics 45:348-354 (1997). This region is frequently associated with the loss of heterozygosity in human colon carcinoma. Leister, I. et al, Human colorectal cancer: high frequency of deletions at chromosome 1p35, Cancer Res. 50:7232-7235 (1990). In the min-mouse tumor model, adenoma multiplicity and growth rate are regulated by APC, the tumor suppressor gene, which is also localized to mouse chromosome 4 in a region syntenic with human chromosome 1p34-36. Dietrich, W.F. et al, Genetic identification of *Mom-1*, a major modifier locus affecting Min-induced intestinal neoplasia in the mouse, Cell 75:631-639 (1992). The APC gene is mutated in the vast majority of humans with colorectal cancer. Miyoshi, Y. et al, Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene, Hum. Mol. Genet. 1:229-233 (1992). The principal function of this gene is to regulate cell cycle via the wnt signal transduction cascade. Cadigan, K.M. et al, Wnt signaling: a common theme in animal development, Genes Dev. 11:3286-3305 (1997). Thus, the uroguanylin and guanylin peptides may be involved early in the process of colon carcinogenesis.

In accordance with the process of the present invention, therefore, a polypeptide which contains the active domain of human uroguanylin or which binds to the



guanylate cyclase receptor GC-C in the intestine of the subject is administered to a subject. While the polypeptide may be administered prophylactically, it will typically be administered to a subject who has been  
5 determined to have intestinal cancer, intestinal polyps, or a genetic predisposition for the growth of polyps in the intestine.

In a preferred embodiment of the present invention, the polypeptide is a polypeptide having the sequence as  
10 identified in SEQ. ID. 1:

X<sub>8</sub>-Asp -Asp -Cys -X<sub>1</sub> -X<sub>2</sub> -Cys -X<sub>3</sub> -Asn -X<sub>4</sub> -X<sub>5</sub> -Cys -X<sub>6</sub>  
-X<sub>7</sub> -Cys-X<sub>9</sub>

15 wherein each of X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub>, X<sub>5</sub>, X<sub>6</sub>, and X<sub>7</sub> is an amino acid residue, X<sub>8</sub> and X<sub>9</sub> are independently hydrogen or at least one amino acid residue, and the polypeptide is cross-linked by a disulfide bond between the cystine residue immediately adjacent the amine group of X<sub>1</sub> and  
20 the cystine residue immediately adjacent the amine group of X<sub>6</sub> and by a disulfide bond between the cystine residue immediately adjacent the amine group of X<sub>3</sub> and the cystine residue immediately adjacent the carboxy group of X<sub>7</sub>. Preferably, the polypeptide is guanylan,  
25 uroguanylin, pro-uroguanylin, or another polypeptide which contains the active domain of uroguanylin.

As is known in the art, certain amino acids in a peptide or protein can be substituted for other amino acids having a similar hydropathic index or score and  
30 produce a resultant peptide or protein having similar biological activity, i.e., which still retains biological functionality. In making such changes, it is preferable that amino acids having hydropathic indices within .2 are substituted for one another. More

preferred substitutions are those wherein the amino acids have hydropathic indices within .1. Most preferred substitutions are those wherein the amino acids have hydropathic indices within .0.5.

5        Like amino acids can also be substituted on the basis of hydrophilicity. U.S. Patent No. 4,554,101 discloses that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological  
10        property of the protein. The following hydrophilicity values have been assigned to amino acids, (according to the Hopp-Woods values): arginine/lysine (+3.0); aspartate/glutamate (+3.0  $\pm$ 1); serine (+0.3); asparagine/glutamine (+0.2); glycine (0); threonine  
15        (-0.4); proline (-0.5  $\pm$ 1); alanine/histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine/isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); and tryptophan (-3.4). Thus, one amino acid in a peptide, polypeptide, or protein can be  
20        substituted by another amino acid having a similar hydrophilicity score and still produce a resultant protein having similar biological activity, i.e., still retaining correct biological function. In making such changes, amino acids having hydropathic indices within  
25         $\pm$ 2 are preferably substituted for one another, those within  $\pm$ 1 are more preferred, and those within  $\pm$ 0.5 are most preferred.

As outlined above, amino acid substitutions in the peptides of the present invention can be based on the  
30        relative similarity of the amino acid side-chain substituents in the non-active domain of the peptide to create a protein with the same biological activity as the human uroguanylin peptide. Thus,  $X_1$  may be selected from the group of all amino acid residues, but

preferably is selected from the group of amino acid residues consisting of aspartic acid, glutamic acid, glycine, lysine, asparagine, proline, glutamine, arginine, serine and threonine. The more preferred amino acid residues that may be substituted for  $X_1$  are glutamic acid, aspartic acid, arginine, and lysine. The most preferred amino acid residue that may be used for  $X_1$  is glutamic acid.  $X_2$  may be selected from all amino acid residues, however the preferred amino acid residues for substitution are leucine, isoleucine, tyrosine, phenylalanine, tryptophan, valine, methionine, cysteine, alanine, histidine, proline, threonine, glycine, asparagine, and glutamine. The more preferred amino acid residues that may be substituted for  $X_2$  are cysteine, phenylalanine, glycine, isoleucine, leucine, methionine, valine, and tyrosine. Among the more preferred amino acid residues mentioned above, the even more preferred amino acid residues for substitution for  $X_2$  are leucine, isoleucine, tyrosine, valine, and methionine. The most preferred amino acid residue for substitution for  $X_2$  is leucine.

Additionally, as discussed above,  $X_3$  and  $X_4$  may be selected from all amino acid residues, but the preferred amino acid residues are valine, isoleucine, tyrosine, phenylalanine, tryptophan, methionine, cysteine, alanine, histidine, proline, threonine, glycine, glutamine, asparagine, and serine. The more preferred amino acid residues that may be substituted for  $X_3$  and  $X_4$  are valine, isoleucine, leucine, tyrosine, phenylalanine, methionine, cysteine, alanine, histidine, and proline. Among the more preferred amino acid residues mentioned above, the even more preferred amino acid residues that may be substituted for  $X_3$  and  $X_4$  are valine, isoleucine, leucine, methionine, and cysteine.

Even more preferable for substitution for X<sub>3</sub> and X<sub>4</sub> are isoleucine and valine. The most preferred amino acid residue for substitution for X<sub>3</sub> and X<sub>4</sub> is valine. Also, X<sub>5</sub> may be selected from all amino acid residues, but the preferred amino acid residues are alanine, histidine, cysteine, methionine, valine, leucine, isoleucine, tyrosine, phenylalanine, proline, threonine, glycine, glutamine, asparagine, and serine. The more preferred amino acid residues that may be substituted for X<sub>5</sub> are alanine, histidine, cysteine, methionine, valine, proline, threonine, glycine, glutamine, asparagine, and serine. Even more preferred amino acid residues for substitution for X<sub>5</sub> are alanine, histidine, cysteine, proline, threonine, glycine, glutamine, asparagine, and serine. The most preferred amino acid residue for substitution for X<sub>5</sub> is alanine.

Moreover, X<sub>6</sub> may be selected from all amino acid residues, but the preferred amino acid residues for substitution are threonine, proline, alanine, histidine, cysteine, methionine, valine, leucine, isoleucine, tyrosine, glycine, glutamine, asparagine, and serine. The more preferred amino acid residues for substitution for X<sub>6</sub> are threonine, proline, alanine, histidine, cysteine, methionine, glycine, glutamine, asparagine, and serine. Even more preferred amino acid residues for substitution are threonine, proline, alanine, histidine, and glycine. The most preferred amino acid residue for substitution for X<sub>6</sub> is threonine. Also, X<sub>7</sub> may be selected from all amino acid residues, but the preferred amino acid residues are glycine, threonine, proline, alanine, histidine, cysteine, methionine, valine, leucine, isoleucine, glutamine, asparagine, serine, glutamic acid, and aspartic acid. The more preferred amino acid residues for substitution for X<sub>7</sub> are glycine,

threonine, proline, alanine, histidine, cysteine, glutamine, asparagine, and serine. Even more preferred amino acid residues for substitution for X<sub>7</sub> are glycine, threonine, proline, alanine, histidine, glutamine, asparagine, and serine. The most preferred amino acid residue for substitution for X<sub>7</sub> is glycine.

The polypeptides and compounds of the present invention can be combined with various excipient vehicles and/or adjuvants well known in this art which serve as pharmaceutically acceptable carriers to permit drug administration in the form of, e.g., injections, suspensions, emulsions, tablets, capsules, and ointments. These pharmaceutical compositions may be administered by any acceptable means. For warm-blooded animals, and in particular, for humans, administration can be oral, parenteral, subcutaneous, intravenous, intramuscular and/or intraperitoneal. The specific dose administered will be dependent upon such factors as the general health and physical condition of the subject as well as the subject's age and weight, the stage of the subject's disease condition, the existence of any concurrent treatments, and the frequency of administration; typically, the dose will be in the range of about 0.5 to about 2.0 mg/kg for human subjects. In general, the composition will contain one or more of the polypeptide(s) of the present invention in a concentration of at least about 0.0001% by weight, more typically at least about 0.001% by weight, still more typically at least about 0.01%, still more typically at least about 0.1% and, in some embodiments, in a concentration of at least about 1% by weight of the composition.

For oral administration, the pharmaceutical composition may be in the form of, for example, a

tablet, capsule, suspension or liquid. The pharmaceutical composition is preferably made in the form of a dosage unit containing a particular amount of the active ingredient. Examples of such dosage units are capsules, tablets, powders, granules or a suspension, with conventional additives such as lactose, mannitol, corn starch or potato starch; with binders such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators such as corn starch, potato starch or sodium carboxymethyl-cellulose; and with lubricants such as talc or magnesium stearate. The active ingredient may also be administered by injection as a composition wherein, for example, saline, dextrose or water may be used as a suitable carrier.

For intravenous, intramuscular, subcutaneous, or intraperitoneal administration, the compound may be combined with a sterile aqueous solution which is preferably isotonic with the blood of the recipient. Such formulations may be prepared by dissolving solid active ingredient in water containing physiologically compatible substances such as sodium chloride, glycine, and the like, and having a buffered pH compatible with physiological conditions to produce an aqueous solution, and rendering said solution sterile. The formulations may be present in unit or multi-dose containers such as sealed ampoules or vials.

If the neoplasia is localized in the G.I. tract, the compound may be formulated with acid-stable, base-labile coatings known in the art which begin to dissolve in the high pH small intestine. Formulation to enhance

local pharmacologic effects and reduce systemic uptake are preferred.

Formulations suitable for parenteral administration conveniently comprise a sterile aqueous preparation of the active compound which is preferably made isotonic. Preparations for injections may also be formulated by suspending or emulsifying the compounds in non-aqueous solvent, such as vegetable oil, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol.

Formulations for topical use include known gels, creams, oils, and the like. For aerosol delivery, the compounds may be formulated with known aerosol excipients, such as saline, and administered using commercially available nebulizers. Formulation in a fatty acid source may be used to enhance biocompatibility. Aerosol delivery is the preferred method of delivery for epithelial neoplasias of the lung for prevention application.

For rectal administration, the active ingredient may be formulated into suppositories using bases which are solid at room temperature and melt or dissolve at body temperature. Commonly used bases include cocoa butter, glycerinated gelatin, hydrogenated vegetable oil, polyethylene glycols of various molecular weights, and fatty esters of polyethylene stearate.

The dosage form and amount can be readily established by reference to known treatment or prophylactic regimens. The amount of therapeutically active compound that is administered and the dosage

regimen for treating a disease condition with the compounds and/or compositions of this invention depends on a variety of factors, including the age, weight, sex and medical condition of the subject, the severity of the disease, the route and frequency of administration, and the particular compound employed, the location of the neoplasia, as well as the pharmacokinetic properties of the individual treated, and thus may vary widely. The dosage will generally be lower if the compounds are administered locally rather than systemically, and for prevention rather than for treatment. Such treatments may be administered as often as necessary and for the period of time judged necessary by the treating physician. One of skill in the art will appreciate that the dosage regime or therapeutically effective amount of the inhibitor to be administered may need to be optimized for each individual. The pharmaceutical compositions may contain active ingredient in the range of about 0.1 to 2000 mg, preferably in the range of about 0.5 to 500 mg and most preferably between about 1 and 200 mg. A daily dose of about 0.01 to 100 mg/kg body weight, preferably between about 0.1 and about 50 mg/kg body weight and most preferably from about 1 to 20 mg/kg body weight, may be appropriate. The daily dose can be administered in one to four doses per day.

Human uroguanylin cDNA has been cloned in bacteria, and chemically synthesized by solid phase peptide synthesis. Uroguanylin peptide can be chemically synthesized by using the procedure as described in U.S. patent number 5,489,670 *Human Uroguanylin* and in U.S. patent number 5,140,102 *Pentadecapeptide, guanylin, which stimulates intestinal guanylate cyclase*. Peptides similar to uroguanylin peptides have been identified in mouse, rat, porcine, and bovine species. The

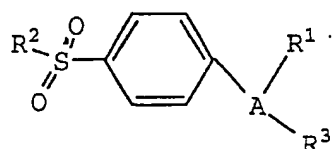


functionally active domain in most of these peptides are highly conserved. Therefore, the physiological functions of these peptides may be similar, and these peptides may be used as intestinal cancer preventative agents as well. Thus, as long as the functionally active domains of these peptides are conserved, substitutions in the non-active domains may be achieved with no change in the activity of the peptides.

In the present invention, the combination of any one or more of the following peptides; uroguanylin, human uroguanylin, pro-uroguanylin, and human pro-uroguanylin, guanylin, lymphoguanylin, prolymphoguanylin and heat stable enterotoxin, with any one of more of naturally occurring, or an extract of a natural occurring, or a chemically synthesized cyclooxygenase-2 inhibitor, preferably a selective cyclooxygenase-2 inhibitor or inhibitors is disclosed for the prevention, inhibition, or treatment of cancer in the intestinal tract by administration of an effective amount of such a combination to a subject in need of such treatment.

In such a combination, the cyclooxygenase inhibitor can be, by way of example, a COX-2 nonselective inhibitor or a COX-2 selective inhibitor. Examples of COX-2 nonselective inhibitors include the well-known compounds aspirin, acetaminophen, indomethacin, sulindac, etodolac, mefenamic acid, tolmetin, ketorolac, diclofenac, ibuprofen, naproxen, fenoprofen, ketoprofen, oxaprozin, flurbiprofen, piroxicam, tenoxicam, phenylbutazone, apazone, or nimesulide or a pharmaceutically acceptable salt or derivative or prodrug thereof. In a preferred embodiment of the invention the COX-2 nonselective inhibitor is selected from the group comprising aspirin, acetaminophen, indomethacin, ibuprofen, or naproxen.

In the preferred embodiments, the cyclooxygenase-2 inhibitor is selected from compounds of Formula I



**I**

5

wherein A is a substituent selected from partially unsaturated or unsaturated heterocyclyl and partially unsaturated or unsaturated carbocyclic rings;

10 wherein R<sup>1</sup> is at least one substituent selected from heterocyclyl, cycloalkyl, cycloalkenyl and aryl, wherein R<sup>1</sup> is optionally substituted at a substitutable position with one or more radicals selected from alkyl, haloalkyl, 15 cyano, carboxyl, alkoxy carbonyl, hydroxyl, hydroxyalkyl, haloalkoxy, amino, alkylamino, arylamino, nitro, alkoxyalkyl, alkylsulfinyl, halo, alkoxy and alkylthio;

wherein R<sup>2</sup> is methyl or amino; and

20 wherein R<sup>3</sup> is a radical selected from hydrido, halo, alkyl, alkenyl, alkynyl, oxo, cyano, carboxyl, cyanoalkyl, heterocycloxy, alkyloxy, alkylthio, alkylcarbonyl, cycloalkyl, aryl, haloalkyl, heterocyclyl, cycloalkenyl, aralkyl, 25 heterocyclylalkyl, acyl, alkylthioalkyl, hydroxyalkyl, alkoxy carbonyl, arylcarbonyl, aralkylcarbonyl, aralkenyl, alkoxyalkyl, arylthioalkyl, aryloxyalkyl, aralkylthioalkyl, aralkoxyalkyl, alkoxyaralkoxyalkyl, 30 alkoxy carbonylalkyl, aminocarbonyl, aminocarbonylalkyl, alkylaminocarbonyl, N-

arylaminocarbonyl, N-alkyl-N-arylaminocarbonyl,  
 alkylaminocarbonylalkyl, carboxyalkyl, alkylamino,  
 N-arylmino, N-aralkylamino, N-alkyl-N-  
 aralkylamino, N-alkyl-N-arylmino, aminoalkyl,  
 5 alkylaminoalkyl, N-arylminoalkyl, N-  
 aralkylaminoalkyl, N-alkyl-N-aralkylaminoalkyl, N-  
 alkyl-N-arylminoalkyl, aryloxy, aralkoxy,  
 arylthio, aralkylthio, alkylsulfinyl,  
 alkylsulfonyl, aminosulfonyl, alkylaminosulfonyl,  
 10 N-arylaminosulfonyl, arylsulfonyl, N-alkyl-N-  
 arylaminosulfonyl; or a pharmaceutically-  
 acceptable salt thereof.

A preferred class of compounds which inhibit  
 cyclooxygenase-2 consists of compounds of Formula I  
 15 wherein A is selected from 5- or 6-member partially  
 unsaturated heterocyclyl, 5- or 6-member  
 unsaturated heterocyclyl, 9- or 10-member  
 unsaturated condensed heterocyclyl, lower  
 cycloalkenyl and phenyl; wherein R<sup>1</sup> is selected  
 20 from 5- and 6-membered heterocyclyl, lower  
 cycloalkyl, lower cycloalkenyl and aryl selected  
 from phenyl, biphenyl and naphthyl, wherein R<sup>1</sup> is  
 optionally substituted at a substitutable position  
 with one or more radicals selected from lower  
 25 alkyl, lower haloalkyl, cyano, carboxyl, lower  
 alkoxy carbonyl, hydroxyl, lower hydroxyalkyl, lower  
 haloalkoxy, amino, lower alkylamino, phenylamino,  
 lower alkoxyalkyl, lower alkylsulfinyl, halo, lower  
 alkoxy and lower alkylthio; wherein R<sup>2</sup> is methyl or  
 30 amino; and wherein R<sup>3</sup> is a radical selected from  
 hydrido, oxo, cyano, carboxyl, lower  
 alkoxy carbonyl, lower carboxyalkyl, lower  
 cyanoalkyl, halo, lower alkyl, lower alkyloxy,  
 lower cycloalkyl, phenyl, lower haloalkyl, 5- or 6-

membered heterocyclyl, lower hydroxylalkyl, lower aralkyl, acyl, phenylcarbonyl, lower alkoxyalkyl, 5- or 6-membered heteroaryloxy, aminocarbonyl, lower alkylaminocarbonyl, lower alkylamino, lower aminoalkyl, lower alkylaminoalkyl, phenoxy, and lower aralkoxy; or a pharmaceutically-acceptable salt thereof.

A more preferred class of compounds which inhibit cyclooxygenase-2 consists of compounds of Formula I wherein A is selected from oxazolyl, isoxazolyl, furyl, thienyl, dihydrofuryl, pyrrolyl, pyrazolyl, thiazolyl, imidazolyl, isothiazolyl, benzofuryl, cyclopentenyl, cyclopentadienyl, phenyl, and pyridyl; wherein R<sup>1</sup> is selected from pyridyl optionally substituted at a substitutable position with one or more methyl radicals, and phenyl optionally substituted at a substitutable position with one or more radicals selected from methyl, ethyl, isopropyl, butyl, tert-butyl, isobutyl, pentyl, hexyl, fluoromethyl, difluoromethyl, trifluoromethyl, cyano, carboxyl, methoxycarbonyl, ethoxycarbonyl, hydroxyl, hydroxymethyl, trifluoromethoxy, amino, N-methylamino, N,N-dimethylamino, N-ethylamino, N,N-dipropylamino, N-butylamino, N-methyl-N-ethylamino, phenylamino, methoxymethyl, methylsulfinyl, fluoro, chloro, bromo, methoxy, ethoxy, propoxy, n-butoxy, pentoxy, and methylthio; wherein R<sup>2</sup> is methyl or amino; and wherein R<sup>3</sup> is a radical selected from hydrido, oxo, cyano, carboxyl, methoxycarbonyl, ethoxycarbonyl, carboxypropyl, carboxymethyl, carboxyethyl, cyanomethyl, fluoro, chloro, bromo, methyl, ethyl, isopropyl, butyl, tert-butyl, isobutyl, pentyl, hexyl, difluoromethyl,

trifluoromethyl, pentafluoroethyl,  
heptafluoropropyl, difluoroethyl, difluoropropyl,  
methoxy, ethoxy, propoxy, n-butoxy, pentoxy,  
cyclohexyl, phenyl, pyridyl, thienyl, thiazolyl,  
5 oxazolyl, furyl, pyrazinyl, hydroxymethyl,  
hydroxylpropyl, benzyl, formyl, phenylcarbonyl,  
methoxymethyl, furylmethyloxy, aminocarbonyl, N-  
methylaminocarbonyl, N,N-dimethylaminocarbonyl,  
N,N-dimethylamino, N-ethylamino, N,N-dipropylamino,  
10 N-butylamino, N-methyl-N-ethylamino, aminomethyl,  
N,N-dimethylaminomethyl, N-methyl-N-  
ethylaminomethyl, benzyloxy, and phenyloxy; or a  
pharmaceutically-acceptable salt thereof.

A family of specific compounds of particular  
15 interest within Formula I consists of compounds  
and pharmaceutically-acceptable salts thereof as  
follows:

5-(4-fluorophenyl)-1-[4-(methylsulfonyl)phenyl]-3-  
(trifluoromethyl)pyrazole;  
20 4-(4-fluorophenyl)-5-[4-(methylsulfonyl)phenyl]-1-  
phenyl-3-(trifluoromethyl)pyrazole;  
4-(5-(4-chlorophenyl)-3-(4-methoxyphenyl)-1H-  
pyrazol-1-yl)benzenesulfonamide  
4-(3,5-bis(4-methylphenyl)-1H-pyrazol-1-  
25 yl)benzenesulfonamide;  
4-(5-(4-chlorophenyl)-3-phenyl-1H-pyrazol-1-  
yl)benzenesulfonamide;  
4-(3,5-bis(4-methoxyphenyl)-1H-pyrazol-1-  
yl)benzenesulfonamide;  
30 4-(5-(4-chlorophenyl)-3-(4-methylphenyl)-1H-pyrazol-  
1-yl)benzenesulfonamide;  
4-(5-(4-chlorophenyl)-3-(4-nitrophenyl)-1H-pyrazol-  
1-yl)benzenesulfonamide;

- 4-(5-(4-chlorophenyl)-3-(5-chloro-2-thienyl)-1H-pyrazol-1-yl)benzenesulfonamide;
- 4-(4-chloro-3,5-diphenyl-1H-pyrazol-1-yl)benzenesulfonamide
- 5 4-[5-(4-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
- 4-[5-phenyl-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
- 4-[5-(4-fluorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
- 10 4-[5-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
- 4-[5-(4-chlorophenyl)-3-(difluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
- 15 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
- 4-[4-chloro-5-(4-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
- 4-[3-(difluoromethyl)-5-(4-methylphenyl)-1H-pyrazol-1-yl]benzenesulfonamide;
- 20 4-[3-(difluoromethyl)-5-phenyl-1H-pyrazol-1-yl]benzenesulfonamide;
- 4-[3-(difluoromethyl)-5-(4-methoxyphenyl)-1H-pyrazol-1-yl]benzenesulfonamide;
- 25 4-[3-cyano-5-(4-fluorophenyl)-1H-pyrazol-1-yl]benzenesulfonamide;
- 4-[3-(difluoromethyl)-5-(3-fluoro-4-methoxyphenyl)-1H-pyrazol-1-yl]benzenesulfonamide;
- 4-[5-(3-fluoro-4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
- 30 4-[4-chloro-5-phenyl-1H-pyrazol-1-yl]benzenesulfonamide;
- 4-[5-(4-chlorophenyl)-3-(hydroxymethyl)-1H-pyrazol-1-yl]benzenesulfonamide;

- 4-[5-(4-(N,N-dimethylamino)phenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
- 5-(4-fluorophenyl)-6-[4-(methylsulfonyl)phenyl]spiro[2.4]hept-5-ene;
- 5 4-[6-(4-fluorophenyl)spiro[2.4]hept-5-en-5-yl]benzenesulfonamide;
- 6-(4-fluorophenyl)-7-[4-(methylsulfonyl)phenyl]spiro[3.4]oct-6-ene;
- 10 5-(3-chloro-4-methoxyphenyl)-6-[4-(methylsulfonyl)phenyl]spiro[2.4]hept-5-ene;
- 4-[6-(3-chloro-4-methoxyphenyl)spiro[2.4]hept-5-en-5-yl]benzenesulfonamide;
- 5-(3,5-dichloro-4-methoxyphenyl)-6-[4-(methylsulfonyl)phenyl]spiro[2.4]hept-5-ene;
- 15 5-(3-chloro-4-fluorophenyl)-6-[4-(methylsulfonyl)phenyl]spiro[2.4]hept-5-ene;
- 4-[6-(3,4-dichlorophenyl)spiro[2.4]hept-5-en-5-yl]benzenesulfonamide;
- 20 2-(3-chloro-4-fluorophenyl)-4-(4-fluorophenyl)-5-(4-methylsulfonylphenyl)thiazole;
- 2-(2-chlorophenyl)-4-(4-fluorophenyl)-5-(4-methylsulfonylphenyl)thiazole;
- 5-(4-fluorophenyl)-4-(4-methylsulfonylphenyl)-2-methylthiazole;
- 25 4-(4-fluorophenyl)-5-(4-methylsulfonylphenyl)-2-trifluoromethylthiazole;
- 4-(4-fluorophenyl)-5-(4-methylsulfonylphenyl)-2-(2-thienyl)thiazole;
- 30 4-(4-fluorophenyl)-5-(4-methylsulfonylphenyl)-2-benzylaminothiazole;
- 4-(4-fluorophenyl)-5-(4-methylsulfonylphenyl)-2-(1-propylamino)thiazole;

- 2-[(3,5-dichlorophenoxy)methyl]-4-(4-fluorophenyl)-  
5-[4-(methylsulfonyl)phenyl]thiazole;  
5-(4-fluorophenyl)-4-(4-methylsulfonylphenyl)-2-  
trifluoromethylthiazole;
- 5 1-methylsulfonyl-4-[1,1-dimethyl-4-(4-  
fluorophenyl)cyclopenta-2,4-dien-3-yl]benzene;  
4-[4-(4-fluorophenyl)-1,1-dimethylcyclopenta-2,4-  
dien-3-yl]benzenesulfonamide;  
5-(4-fluorophenyl)-6-[4-
- 10 (methylsulfonyl)phenyl]spiro[2.4]hepta-4,6-diene;  
4-[6-(4-fluorophenyl)spiro[2.4]hepta-4,6-dien-5-  
yl]benzenesulfonamide;  
6-(4-fluorophenyl)-2-methoxy-5-[4-  
(methylsulfonyl)phenyl]-pyridine-3-carbonitrile;
- 15 2-bromo-6-(4-fluorophenyl)-5-[4-  
(methylsulfonyl)phenyl]-pyridine-3-carbonitrile;  
6-(4-fluorophenyl)-5-[4-(methylsulfonyl)phenyl]-2-  
phenylpyridine-3-carbonitrile;  
4-[2-(4-methylpyridin-2-yl)-4-(trifluoromethyl)-1H-
- 20 imidazol-1-yl]benzenesulfonamide;  
4-[2-(5-methylpyridin-3-yl)-4-(trifluoromethyl)-1H-  
imidazol-1-yl]benzenesulfonamide;  
4-[2-(2-methylpyridin-3-yl)-4-(trifluoromethyl)-1H-  
imidazol-1-yl]benzenesulfonamide;
- 25 3-[1-[4-(methylsulfonyl)phenyl]-4-(trifluoromethyl)-  
1H-imidazol-2-yl]pyridine;  
2-[1-[4-(methylsulfonyl)phenyl]-4-(trifluoromethyl)-  
1H-imidazol-2-yl]pyridine;  
2-methyl-4-[1-[4-(methylsulfonyl)phenyl]-4-
- 30 (trifluoromethyl)-1H-imidazol-2-yl]pyridine;  
2-methyl-6-[1-[4-(methylsulfonyl)phenyl]-4-  
(trifluoromethyl)-1H-imidazol-2-yl]pyridine;  
4-[2-(6-methylpyridin-3-yl)-4-(trifluoromethyl)-1H-  
imidazol-1-yl]benzenesulfonamide;



- 2-(3,4-difluorophenyl)-1-[4-(methylsulfonyl)phenyl]-  
4-(trifluoromethyl)-1H-imidazole;  
4-[2-(4-methylphenyl)-4-(trifluoromethyl)-1H-  
imidazol-1-yl]benzenesulfonamide;
- 5 2-(4-chlorophenyl)-1-[4-(methylsulfonyl)phenyl]-4-  
methyl-1H-imidazole;  
2-(4-chlorophenyl)-1-[4-(methylsulfonyl)phenyl]-4-  
phenyl-1H-imidazole;  
2-(4-chlorophenyl)-4-(4-fluorophenyl)-1-[4-  
10 (methylsulfonyl)phenyl]-1H-imidazole;  
2-(3-fluoro-4-methoxyphenyl)-1-[4-  
(methylsulfonyl)phenyl]-4-(trifluoromethyl)-1H-  
imidazole;  
1-[4-(methylsulfonyl)phenyl]-2-phenyl-4-  
15 trifluoromethyl-1H-imidazole;  
2-(4-methylphenyl)-1-[4-(methylsulfonyl)phenyl]-4-  
trifluoromethyl-1H-imidazole;  
4-[2-(3-chloro-4-methylphenyl)-4-(trifluoromethyl)-  
1H-imidazol-1-yl]benzenesulfonamide;
- 20 2-(3-fluoro-5-methylphenyl)-1-[4-  
(methylsulfonyl)phenyl]-4-(trifluoromethyl)-1H-  
imidazole;  
4-[2-(3-fluoro-5-methylphenyl)-4-(trifluoromethyl)-  
1H-imidazol-1-yl]benzenesulfonamide;
- 25 2-(3-methylphenyl)-1-[4-(methylsulfonyl)phenyl]-4-  
trifluoromethyl-1H-imidazole;  
4-[2-(3-methylphenyl)-4-trifluoromethyl-1H-imidazol-  
1-yl]benzenesulfonamide;
- 30 1-[4-(methylsulfonyl)phenyl]-2-(3-chlorophenyl)-4-  
trifluoromethyl-1H-imidazole;  
4-[2-(3-chlorophenyl)-4-trifluoromethyl-1H-imidazol-  
1-yl]benzenesulfonamide;  
4-[2-phenyl-4-trifluoromethyl-1H-imidazol-1-  
yl]benzenesulfonamide;

- 4-[2-(4-methoxy-3-chlorophenyl)-4-trifluoromethyl-  
1H-imidazol-1-yl]benzenesulfonamide;  
1-allyl-4-(4-fluorophenyl)-3-[4-  
(methylsulfonyl)phenyl]-5-(trifluoromethyl)-1H-  
5 pyrazole;  
4-[1-ethyl-4-(4-fluorophenyl)-5-(trifluoromethyl)-  
1H-pyrazol-3-yl]benzenesulfonamide;  
N-phenyl-[4-(4-fluorophenyl)-3-[4-  
(methylsulfonyl)phenyl]-5-(trifluoromethyl)-1H-  
10 pyrazol-1-yl]acetamide;  
ethyl [4-(4-fluorophenyl)-3-[4-  
(methylsulfonyl)phenyl]-5-(trifluoromethyl)-1H-  
pyrazol-1-yl]acetate;  
4-(4-fluorophenyl)-3-[4-(methylsulfonyl)phenyl]-1-  
15 (2-phenylethyl)-1H-pyrazole;  
4-(4-fluorophenyl)-3-[4-(methylsulfonyl)phenyl]-1-  
(2-phenylethyl)-5-(trifluoromethyl)pyrazole;  
1-ethyl-4-(4-fluorophenyl)-3-[4-  
(methylsulfonyl)phenyl]-5-(trifluoromethyl)-1H-  
20 pyrazole;  
5-(4-fluorophenyl)-4-(4-methylsulfonylphenyl)-2-  
trifluoromethyl-1H-imidazole;  
4-[4-(methylsulfonyl)phenyl]-5-(2-thiophenyl)-2-  
(trifluoromethyl)-1H-imidazole;  
25 5-(4-fluorophenyl)-2-methoxy-4-[4-  
(methylsulfonyl)phenyl]-6-  
(trifluoromethyl)pyridine;  
2-ethoxy-5-(4-fluorophenyl)-4-[4-  
(methylsulfonyl)phenyl]-6-  
30 (trifluoromethyl)pyridine;  
5-(4-fluorophenyl)-4-[4-(methylsulfonyl)phenyl]-2-  
(2-propynyloxy)-6-(trifluoromethyl)pyridine;

- 2-bromo-5-(4-fluorophenyl)-4-[4-(methylsulfonyl)phenyl]-6-(trifluoromethyl)pyridine;  
4-[2-(3-chloro-4-methoxyphenyl)-4,5-difluorophenyl]benzenesulfonamide;  
5 1-(4-fluorophenyl)-2-[4-(methylsulfonyl)phenyl]benzene;  
5-difluoromethyl-4-(4-methylsulfonylphenyl)-3-phenylisoxazole;  
10 4-[3-ethyl-5-phenylisoxazol-4-yl]benzenesulfonamide;  
4-[5-difluoromethyl-3-phenylisoxazol-4-yl]benzenesulfonamide;  
4-[5-hydroxymethyl-3-phenylisoxazol-4-yl]benzenesulfonamide;  
15 4-[5-methyl-3-phenyl-isoxazol-4-yl]benzenesulfonamide;  
1-[2-(4-fluorophenyl)cyclopenten-1-yl]-4-(methylsulfonyl)benzene;  
1-[2-(4-fluoro-2-methylphenyl)cyclopenten-1-yl]-4-(methylsulfonyl)benzene;  
20 1-[2-(4-chlorophenyl)cyclopenten-1-yl]-4-(methylsulfonyl)benzene;  
1-[2-(2,4-dichlorophenyl)cyclopenten-1-yl]-4-(methylsulfonyl)benzene;  
25 1-[2-(4-trifluoromethylphenyl)cyclopenten-1-yl]-4-(methylsulfonyl)benzene;  
1-[2-(4-methylthiophenyl)cyclopenten-1-yl]-4-(methylsulfonyl)benzene;  
1-[2-(4-fluorophenyl)-4,4-dimethylcyclopenten-1-yl]-4-(methylsulfonyl)benzene;  
30 4-[2-(4-fluorophenyl)-4,4-dimethylcyclopenten-1-yl]benzenesulfonamide;  
1-[2-(4-chlorophenyl)-4,4-dimethylcyclopenten-1-yl]-4-(methylsulfonyl)benzene;

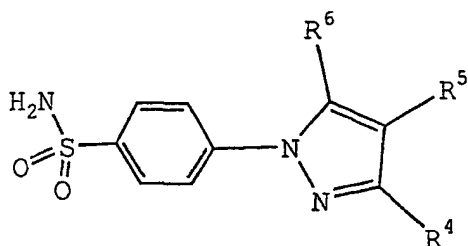
- 4-[2-(4-chlorophenyl)-4,4-dimethylcyclopenten-1-yl]benzenesulfonamide;  
4-[2-(4-fluorophenyl)cyclopenten-1-yl]benzenesulfonamide;  
5 4-[2-(4-chlorophenyl)cyclopenten-1-yl]benzenesulfonamide;  
1-[2-(4-methoxyphenyl)cyclopenten-1-yl]-4-(methylsulfonyl)benzene;  
1-[2-(2,3-difluorophenyl)cyclopenten-1-yl]-4-(methylsulfonyl)benzene;  
10 4-[2-(3-fluoro-4-methoxyphenyl)cyclopenten-1-yl]benzenesulfonamide;  
1-[2-(3-chloro-4-methoxyphenyl)cyclopenten-1-yl]-4-(methylsulfonyl)benzene;  
15 4-[2-(3-chloro-4-fluorophenyl)cyclopenten-1-yl]benzenesulfonamide;  
4-[2-(2-methylpyridin-5-yl)cyclopenten-1-yl]benzenesulfonamide;  
ethyl 2-[4-(4-fluorophenyl)-5-[4-(methylsulfonyl)phenyl]oxazol-2-yl]-2-benzyl-acetate;  
20 2-[4-(4-fluorophenyl)-5-[4-(methylsulfonyl)phenyl]oxazol-2-yl]acetic acid;  
2-(tert-butyl)-4-(4-fluorophenyl)-5-[4-(methylsulfonyl)phenyl]oxazole;  
25 4-(4-fluorophenyl)-5-[4-(methylsulfonyl)phenyl]-2-phenyloxazole;  
4-(4-fluorophenyl)-2-methyl-5-[4-(methylsulfonyl)phenyl]oxazole; and  
4-[5-(3-fluoro-4-methoxyphenyl)-2-trifluoromethyl-4-oxazolyl]benzenesulfonamide.  
30

A family of specific compounds of more particular interest within Formula I consists of compounds and pharmaceutically-acceptable salts thereof as follows:

- 4-[5-(4-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;  
4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;  
5 4-[5-(3-fluoro-4-methoxyphenyl)-3-(difluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;  
3-[1-[4-(methylsulfonyl)phenyl]-4-trifluoromethyl-1H-imidazol-2-yl]pyridine;  
2-methyl-5-[1-[4-(methylsulfonyl)phenyl]-4-trifluoromethyl-1H-imidazol-2-yl]pyridine;  
10 4-[2-(5-methylpyridin-3-yl)-4-(trifluoromethyl)-1H-imidazol-1-yl]benzenesulfonamide;  
4-[5-methyl-3-phenylisoxazol-4-yl]benzenesulfonamide;  
15 4-[5-hydroxymethyl-3-phenylisoxazol-4-yl]benzenesulfonamide;  
[2-trifluoromethyl-5-(3,4-difluorophenyl)-4-oxazolyl]benzenesulfonamide;  
4-[2-methyl-4-phenyl-5-oxazolyl]benzenesulfonamide;  
20 and  
4-[5-(3-fluoro-4-methoxyphenyl-2-trifluoromethyl)-4-oxazolyl]benzenesulfonamide.

A subclass of cyclooxygenase-2 inhibitors is selected from compounds of Formula II

25

**II**

wherein R<sup>4</sup> is selected from hydrido, alkyl, haloalkyl, alkoxy carbonyl, cyano, cyanoalkyl, carboxyl, aminocarbonyl, alkylaminocarbonyl, cycloalkylaminocarbonyl, arylaminocarbonyl, carboxyalkylaminocarbonyl, carboxyalkyl, aralkoxy carbonyl alkylaminocarbonyl, aminocarbonyl alkyl, alkoxy carbonyl cyanoalkenyl and hydroxyalkyl;

wherein R<sup>5</sup> is selected from hydrido, alkyl, cyano, hydroxyalkyl, cycloalkyl, alkylsulfonyl and halo; and

10 wherein R<sup>6</sup> is selected from aralkenyl, aryl, cycloalkyl, cycloalkenyl and heterocyclic; wherein R<sup>4</sup> is optionally substituted at a substitutable position with one or more radicals selected from halo, alkylthio, alkylsulfonyl, cyano, nitro, haloalkyl, alkyl, hydroxyl, alkenyl, hydroxyalkyl, carboxyl, cycloalkyl, alkylamino, 15 dialkylamino, alkoxy carbonyl, aminocarbonyl, alkoxy, haloalkoxy, sulfamyl, heterocyclic and amino;

or a pharmaceutically-acceptable salt or derivative thereof.

20 A class of compounds of particular interest consists of those compounds of Formula I wherein R<sup>4</sup> is selected from hydrido, lower alkyl, lower haloalkyl, lower alkoxy carbonyl, cyano, lower cyanoalkyl, carboxyl, aminocarbonyl, lower alkylaminocarbonyl, lower cycloalkylaminocarbonyl, arylaminocarbonyl, lower 25 carboxyalkylaminocarbonyl, lower aminocarbonyl alkyl, lower aralkoxy carbonyl alkylaminocarbonyl, lower carboxyalkyl, lower alkoxy carbonyl cyanoalkenyl and lower hydroxyalkyl; wherein R<sup>5</sup> is selected from hydrido, 30 lower alkyl, cyano, lower hydroxyalkyl, lower

cycloalkyl, lower alkylsulfonyl and halo; and wherein R<sup>6</sup> is selected from aralkenyl, aryl, cycloalkyl, cycloalkenyl and heterocyclic; wherein R<sup>4</sup> is optionally substituted at a substitutable position with one or  
5 more radicals selected from halo, lower alkylthio, lower alkylsulfonyl, cyano, nitro, lower haloalkyl, lower alkyl, hydroxyl, lower alkenyl, lower hydroxyalkyl, carboxyl, lower cycloalkyl, lower alkylamino, lower dialkylamino, lower alkoxy carbonyl, aminocarbonyl, lower  
10 alkoxy, lower haloalkoxy, sulfamyl, five or six membered heterocyclic and amino; or a pharmaceutically-acceptable salt or derivative thereof.

A family of specific compounds of particular interest within Formula I consists of compounds,  
15 derivatives and pharmaceutically-acceptable salts thereof as follows:

4-[5-(4-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;  
4-[5-phenyl-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;  
20 4-[5-(4-fluorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;  
4-[5-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;  
25 4-[5-(4-chlorophenyl)-3-(difluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;  
4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;  
4-[4-chloro-5-(4-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;  
30 4-[3-(difluoromethyl)-5-(4-methylphenyl)-1H-pyrazol-1-yl]benzenesulfonamide;  
4-[3-(difluoromethyl)-5-phenyl-1H-pyrazol-1-yl]benzenesulfonamide;

- 4-[3-(difluoromethyl)-5-(4-methoxyphenyl)-1H-pyrazol-1-yl]benzenesulfonamide;  
4-[3-cyano-5-(4-fluorophenyl)-1H-pyrazol-1-yl]benzenesulfonamide;  
5 4-[3-(difluoromethyl)-5-(3-fluoro-4-methoxyphenyl)-1H-pyrazol-1-yl]benzenesulfonamide;  
4-[5-(3-fluoro-4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;  
4-[4-chloro-5-phenyl-1H-pyrazol-1-yl]benzenesulfonamide;  
10 4-[5-(4-chlorophenyl)-3-(hydroxymethyl)-1H-pyrazol-1-yl]benzenesulfonamide; and  
4-[5-(4-(N,N-dimethylamino)phenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide.

15 A family of specific compounds of more particular interest within Formula I consists of compounds and pharmaceutically-acceptable salts or derivatives thereof as follows:

- 20 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;  
4-[5-(4-chlorophenyl)-3-(difluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide; and  
4-[5-(3-fluoro-4-methoxyphenyl)-3-(difluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide.  
25

Derivatives are intended to encompass any compounds which are structurally related to the cyclooxygenase-2 inhibitors or which possess the substantially equivalent biologic activity. By way of example, such inhibitors  
30 may include, but are not limited to, prodrugs thereof.

The term "hydrido" denotes a single hydrogen atom (H). This hydrido radical may be attached,



for example, to an oxygen atom to form a hydroxyl radical or two hydrido radicals may be attached to a carbon atom to form a methylene ( $-\text{CH}_2-$ ) radical. Where used, either alone or within  
5 other terms such as "haloalkyl", "alkylsulfonyl", "alkoxyalkyl" and "hydroxyalkyl", the term "alkyl" embraces linear or branched radicals having one to about twenty carbon atoms or, preferably, one to about twelve carbon atoms.  
10 More preferred alkyl radicals are "lower alkyl" radicals having one to about ten carbon atoms. Most preferred are lower alkyl radicals having one to about six carbon atoms. Examples of such radicals include methyl, ethyl, n-propyl,  
15 isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, iso-amyl, hexyl and the like. The term "alkenyl" embraces linear or branched radicals having at least one carbon-carbon double bond of two to about twenty carbon atoms or,  
20 preferably, two to about twelve carbon atoms. More preferred alkyl radicals are "lower alkenyl" radicals having two to about six carbon atoms. Examples of alkenyl radicals include ethenyl, propenyl, allyl, propenyl, butenyl and 4-methylbutenyl.  
25 The term "alkynyl" denotes linear or branched radicals having two to about twenty carbon atoms or, preferably, two to about twelve carbon atoms. More preferred alkynyl radicals are "lower alkynyl" radicals having two to about ten  
30 carbon atoms. Most preferred are lower alkynyl radicals having two to about six carbon atoms. Examples of such radicals include propargyl, butynyl, and the like. The terms "alkenyl", "lower alkenyl", embrace radicals having "cis"

and "trans" orientations, or alternatively, "E" and "Z" orientations. The term "cycloalkyl" embraces saturated carbocyclic radicals having three to twelve carbon atoms. More preferred cycloalkyl radicals are "lower cycloalkyl" radicals having three to about eight carbon atoms. Examples of such radicals include cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. The term "cycloalkenyl" embraces partially unsaturated carbocyclic radicals having three to twelve carbon atoms. More preferred cycloalkenyl radicals are "lower cycloalkenyl" radicals having four to about eight carbon atoms. Examples of such radicals include cyclobutenyl, cyclopentenyl, cyclopentadienyl, and cyclohexenyl. The term "halo" means halogens such as fluorine, chlorine, bromine or iodine. The term "haloalkyl" embraces radicals wherein any one or more of the alkyl carbon atoms is substituted with halo as defined above. Specifically embraced are monohaloalkyl, dihaloalkyl and polyhaloalkyl radicals. A monohaloalkyl radical, for one example, may have either an iodo, bromo, chloro or fluoro atom within the radical. Dihalo and polyhaloalkyl radicals may have two or more of the same halo atoms or a combination of different halo radicals. "Lower haloalkyl" embraces radicals having 1-6 carbon atoms. Examples of haloalkyl radicals include fluoromethyl, difluoromethyl, trifluoromethyl, chloromethyl, dichloromethyl, trichloromethyl, trichloromethyl, pentafluoroethyl, heptafluoropropyl, difluorochloromethyl, dichlorofluoromethyl,

difluoroethyl, difluoropropyl, dichloroethyl and dichloropropyl. The term "hydroxyalkyl" embraces linear or branched alkyl radicals having one to about ten carbon atoms any one of which may be substituted with one or more hydroxyl radicals. More preferred hydroxyalkyl radicals are "lower hydroxyalkyl" radicals having one to six carbon atoms and one or more hydroxyl radicals. Examples of such radicals include hydroxymethyl, hydroxyethyl, hydroxypropyl, hydroxybutyl and hydroxyhexyl. The terms "alkoxy" and "alkyloxy" embrace linear or branched alkoxy-containing radicals each having alkyl portions of one to about ten carbon atoms. More preferred alkoxy radicals are "lower alkoxy" radicals having one to six carbon atoms. Examples of such radicals include methoxy, ethoxy, propoxy, butoxy and tert-butoxy. The term "alkoxyalkyl" embraces alkyl radicals having one or more alkoxy radicals attached to the alkyl radical, that is, to form monoalkoxyalkyl and dialkoxyalkyl radicals. The "alkoxy" radicals may be further substituted with one or more halo atoms, such as fluoro, chloro or bromo, to provide haloalkoxy radicals. More preferred haloalkoxy radicals are "lower haloalkoxy" radicals having one to six carbon atoms and one or more halo radicals. Examples of such radicals include fluoromethoxy, chloromethoxy, trifluoromethoxy, trifluoroethoxy, fluoroethoxy and fluoropropoxy. The term "aryl", alone or in combination, means a carbocyclic aromatic system containing one, two or three rings wherein such rings may be attached together in a pendent manner or may be fused. The term

"aryl" embraces aromatic radicals such as phenyl, naphthyl, tetrahydronaphthyl, indane and biphenyl. Aryl moieties may also be substituted at a substitutable position with one or more substituents selected independently from alkyl, alkoxyalkyl, alkylaminoalkyl, carboxyalkyl, alkoxycarbonylalkyl, aminocarbonylalkyl, alkoxy, aralkoxy, hydroxyl, amino, halo, nitro, alkylamino, acyl, cyano, carboxy, aminocarbonyl, alkoxycarbonyl and aralkoxycarbonyl. The term "heterocyclyl" embraces saturated, partially unsaturated and unsaturated heteroatom-containing ring-shaped radicals, where the heteroatoms may be selected from nitrogen, sulfur and oxygen. Examples of saturated heterocyclyl radicals include saturated 3 to 6-membered heteromonocyclic group containing 1 to 4 nitrogen atoms (e.g. pyrrolidinyl, imidazolidinyl, piperidino, piperazinyl, etc.); saturated 3 to 6-membered heteromonocyclic group containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms (e.g. morpholinyl, etc.); saturated 3 to 6-membered heteromonocyclic group containing 1 to 2 sulfur atoms and 1 to 3 nitrogen atoms (e.g., thiazolidinyl, etc.). Examples of partially unsaturated heterocyclyl radicals include dihydrothiophene, dihydropyran, dihydrofuran and dihydrothiazole. The term "heteroaryl" embraces unsaturated heterocyclyl radicals. Examples of unsaturated heterocyclyl radicals, also termed "heteroaryl" radicals include unsaturated 3 to 6 membered heteromonocyclic group containing 1 to 4 nitrogen atoms, for example, pyrrolyl, pyrrolinyl, imidazolyl, pyrazolyl, pyridyl,

pyrimidyl, pyrazinyl, pyridazinyl, triazolyl (e.g., 4H-1,2,4-triazolyl, 1H-1,2,3-triazolyl, 2H-1,2,3-triazolyl, etc.) tetrazolyl (e.g. 1H-tetrazolyl, 2H-tetrazolyl, etc.), etc.;

5 unsaturated condensed heterocyclyl group containing 1 to 5 nitrogen atoms, for example, indolyl, isoindolyl, indoliziny, benzimidazolyl, quinolyl, isoquinolyl, indazolyl, benzotriazolyl, tetrazolopyridazinyl (e.g., tetrazolo[1,5-

10 b]pyridazinyl, etc.), etc.; unsaturated 3 to 6-membered heteromonocyclic group containing an oxygen atom, for example, pyranyl, furyl, etc.; unsaturated 3 to 6-membered heteromonocyclic group containing a sulfur atom, for example,

15 thienyl, etc.; unsaturated 3- to 6-membered heteromonocyclic group containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms, for example, oxazolyl, isoxazolyl, oxadiazolyl (e.g., 1,2,4-oxadiazolyl, 1,3,4-oxadiazolyl, 1,2,5-

20 oxadiazolyl, etc.) etc.; unsaturated condensed heterocyclyl group containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms (e.g. benzoxazolyl, benzoxadiazolyl, etc.); unsaturated 3 to 6-membered heteromonocyclic group containing 1 to 2

25 sulfur atoms and 1 to 3 nitrogen atoms, for example, thiazolyl, thiadiazolyl (e.g., 1,2,4-thiadiazolyl, 1,3,4-thiadiazolyl, 1,2,5-thiadiazolyl, etc.) etc.; unsaturated condensed heterocyclyl group containing 1 to 2 sulfur atoms

30 and 1 to 3 nitrogen atoms (e.g., benzothiazolyl, benzothiadiazolyl, etc.) and the like. The term also embraces radicals where heterocyclyl radicals are fused with aryl radicals. Examples of such fused bicyclic radicals include

benzofuran, benzothiophene, and the like. Said "heterocyclyl group" may have 1 to 3 substituents such as alkyl, hydroxyl, halo, alkoxy, oxo, amino and alkylamino. The term "alkylthio" embraces radicals containing a linear or branched alkyl radical, of one to about ten carbon atoms attached to a divalent sulfur atom. More preferred alkylthio radicals are "lower alkylthio" radicals having alkyl radicals of one to six carbon atoms. Examples of such lower alkylthio radicals are methylthio, ethylthio, propylthio, butylthio and hexylthio. The term "alkylthioalkyl" embraces radicals containing an alkylthio radical attached through the divalent sulfur atom to an alkyl radical of one to about ten carbon atoms. More preferred alkylthioalkyl radicals are "lower alkylthioalkyl" radicals having alkyl radicals of one to six carbon atoms. Examples of such lower alkylthioalkyl radicals include methylthiomethyl. The term "alkylsulfinyl" embraces radicals containing a linear or branched alkyl radical, of one to ten carbon atoms, attached to a divalent  $-S(=O)-$  radical. More preferred alkylsulfinyl radicals are "lower alkylsulfinyl" radicals having alkyl radicals of one to six carbon atoms. Examples of such lower alkylsulfinyl radicals include methylsulfinyl, ethylsulfinyl, butylsulfinyl and hexylsulfinyl. The term "sulfonyl", whether used alone or linked to other terms such as alkylsulfonyl, denotes respectively divalent radicals  $-SO_2-$ . "Alkylsulfonyl" embraces alkyl radicals attached to a sulfonyl radical, where alkyl is defined as above. More preferred

alkylsulfonyl radicals are "lower alkylsulfonyl" radicals having one to six carbon atoms. Examples of such lower alkylsulfonyl radicals include methylsulfonyl, ethylsulfonyl and propylsulfonyl. The "alkylsulfonyl" radicals may be further substituted with one or more halo atoms, such as fluoro, chloro or bromo, to provide haloalkylsulfonyl radicals. The terms "sulfamyl", "aminosulfonyl" and "sulfonamidyl" denote  $\text{NH}_2\text{O}_2\text{S}-$ . The term "acyl" denotes a radical provided by the residue after removal of hydroxyl from an organic acid. Examples of such acyl radicals include alkanoyl and aroyl radicals. Examples of such lower alkanoyl radicals include formyl, acetyl, propionyl, butyryl, isobutyryl, valeryl, isovaleryl, pivaloyl, hexanoyl, trifluoroacetyl. The term "carbonyl", whether used alone or with other terms, such as "alkoxycarbonyl", denotes  $-(\text{C}=\text{O})-$ . The term "aroyl" embraces aryl radicals with a carbonyl radical as defined above. Examples of aroyl include benzoyl, naphthoyl, and the like and the aryl in said aroyl may be additionally substituted. The terms "carboxy" or "carboxyl", whether used alone or with other terms, such as "carboxyalkyl", denotes  $-\text{CO}_2\text{H}$ . The term "carboxyalkyl" embraces alkyl radicals substituted with a carboxy radical. More preferred are "lower carboxyalkyl" which embrace lower alkyl radicals as defined above, and may be additionally substituted on the alkyl radical with halo. Examples of such lower carboxyalkyl radicals include carboxymethyl, carboxyethyl and carboxypropyl. The term "alkoxycarbonyl" means a

radical containing an alkoxy radical, as defined above, attached via an oxygen atom to a carbonyl radical. More preferred are "lower alkoxy carbonyl" radicals with alkyl portions having 1 to 6 carbons. Examples of such lower alkoxy carbonyl (ester) radicals include substituted or unsubstituted methoxy carbonyl, ethoxy carbonyl, propoxy carbonyl, butoxy carbonyl and hexyloxy carbonyl. The terms "alkyl carbonyl", "aryl carbonyl" and "aralkyl carbonyl" include radicals having alkyl, aryl and aralkyl radicals, as defined above, attached to a carbonyl radical. Examples of such radicals include substituted or unsubstituted methyl carbonyl, ethyl carbonyl, phenyl carbonyl and benzyl carbonyl. The term "aralkyl" embraces aryl-substituted alkyl radicals such as benzyl, diphenylmethyl, triphenylmethyl, phenylethyl, and diphenylethyl. The aryl in said aralkyl may be additionally substituted with halo, alkyl, alkoxy, haloalkyl and haloalkoxy. The terms benzyl and phenylmethyl are interchangeable. The term "heterocyclyl alkyl" embraces saturated and partially unsaturated heterocyclyl-substituted alkyl radicals, such as pyrrolidinylmethyl, and heteroaryl-substituted alkyl radicals, such as pyridylmethyl, quinolylmethyl, thienylmethyl, furylethyl, and quinolyethylethyl. The heteroaryl in said heteroaralkyl may be additionally substituted with halo, alkyl, alkoxy, haloalkyl and haloalkoxy. The term "aralkoxy" embraces aralkyl radicals attached through an oxygen atom to other radicals. The term "aralkoxy alkyl" embraces aralkoxy radicals attached through an



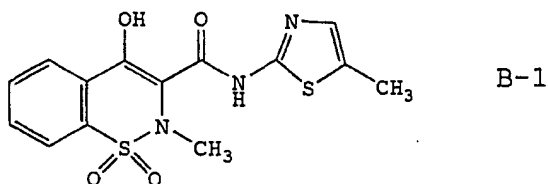
oxygen atom to an alkyl radical. The term "aralkylthio" embraces aralkyl radicals attached to a sulfur atom. The term "aralkylthioalkyl" embraces aralkylthio radicals attached through a sulfur atom to an alkyl radical. The term "aminoalkyl" embraces alkyl radicals substituted with one or more amino radicals. More preferred are "lower aminoalkyl" radicals. Examples of such radicals include aminomethyl, aminoethyl, and the like. The term "alkylamino" denotes amino groups which have been substituted with one or two alkyl radicals. Preferred are "lower N-alkylamino" radicals having alkyl portions having 1 to 6 carbon atoms. Suitable lower alkylamino may be mono or dialkylamino such as N-methylamino, N-ethylamino, N,N-dimethylamino, N,N-diethylamino or the like. The term "arylamino" denotes amino groups which have been substituted with one or two aryl radicals, such as N-phenylamino. The "arylamino" radicals may be further substituted on the aryl ring portion of the radical. The term "aralkylamino" embraces aralkyl radicals attached through an amino nitrogen atom to other radicals. The terms "N-arylaminoalkyl" and "N-aryl-N-alkyl-aminoalkyl" denote amino groups which have been substituted with one aryl radical or one aryl and one alkyl radical, respectively, and having the amino group attached to an alkyl radical. Examples of such radicals include N-phenylaminomethyl and N-phenyl-N-methylaminomethyl. The term "aminocarbonyl" denotes an amide group of the formula  $-C(=O)NH_2$ . The term "alkylaminocarbonyl" denotes an aminocarbonyl group which has been

substituted with one or two alkyl radicals on the amino nitrogen atom. Preferred are "N-alkylaminocarbonyl" "N,N-dialkylaminocarbonyl" radicals. More preferred are "lower N-alkylaminocarbonyl" "lower N,N-dialkylaminocarbonyl" radicals with lower alkyl portions as defined above. The term "alkylaminoalkyl" embraces radicals having one or more alkyl radicals attached to an aminoalkyl radical. The term "aryloxyalkyl" embraces radicals having an aryl radical attached to an alkyl radical through a divalent oxygen atom. The term "arylthioalkyl" embraces radicals having an aryl radical attached to an alkyl radical through a divalent sulfur atom.

The compounds utilized in the methods of the present invention may be present in the form of free bases or pharmaceutically acceptable acid addition salts thereof. The term "pharmaceutically-acceptable salts" embraces salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases. The nature of the salt is not critical, provided that it is pharmaceutically-acceptable. Suitable pharmaceutically-acceptable acid addition salts of compounds of Formula I may be prepared from an inorganic acid or from an organic acid. Examples of such inorganic acids are hydrochloric, hydrobromic, hydroiodic, nitric, carbonic, sulfuric and phosphoric acid. Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, example of which are formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic,

aspartic, glutamic, benzoic, anthranilic, mesylic, 4-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, 2-hydroxyethanesulfonic, 5 toluenesulfonic, sulfanilic, cyclohexylaminosulfonic, stearic, algenic, D-3-hydroxybutyric, salicylic, galactaric and galacturonic acid. Suitable pharmaceutically-acceptable base addition salts of compounds of Formula I include metallic salts made from 10 aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. All of these salts may 15 be prepared by conventional means from the corresponding compound of Formula I by reacting, for example, the appropriate acid or base with the compound of Formula I.

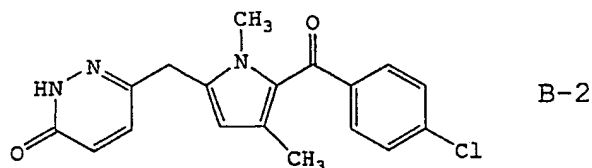
In another embodiment of the invention the cyclooxygenase inhibitor can be a cyclooxygenase-2 20 selective inhibitor, for example, the COX-2 selective inhibitor meloxicam, Formula B-1 (CAS registry number 71125-38-7) or a pharmaceutically acceptable salt or derivative or prodrug thereof.



In yet another embodiment of the invention the cyclooxygenase-2 selective inhibitor is the COX-2 selective inhibitor RS 57067, 6-[[5-(4-chlorobenzoyl)- 30 1,4-dimethyl-1H-pyrrol-2-yl)methyl]-3(2H)-pyridazinone,

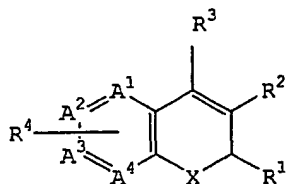
Formula B-2 (CAS registry number 179382-91-3) or a pharmaceutically acceptable salt or derivative or prodrug thereof.

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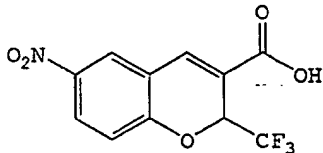
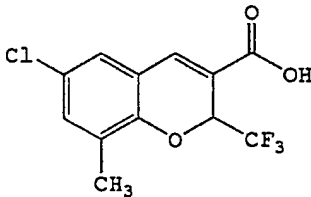
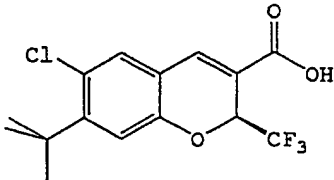
In a preferred embodiment of the invention the  
10 cyclooxygenase-2 selective inhibitor is a COX-2  
selective inhibitor of the chromene structural class  
that is a substituted benzopyran or a substituted  
benzopyran analog selected from the group consisting of  
substituted benzothiopyrans, dihydroquinolines, or  
15 dihydronaphthalenes having the general Formula II shown  
below and possessing, by way of example and not  
limitation, the structures disclosed in Table 1,  
including the diastereomers, enantiomers, racemates,  
tautomers, salts, esters, amides and prodrugs thereof.

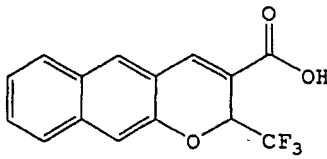
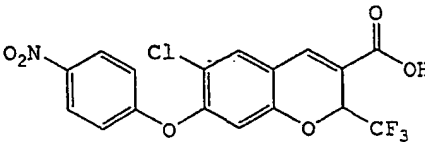
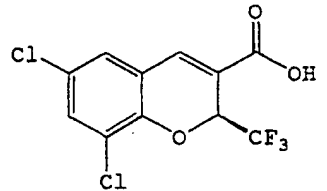
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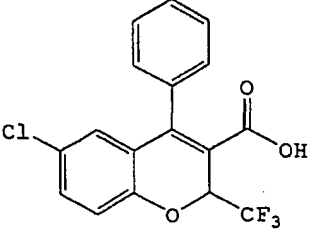
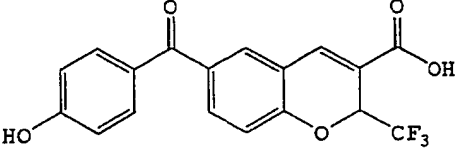
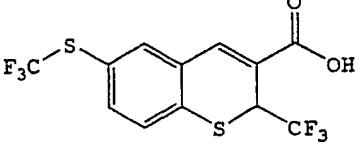


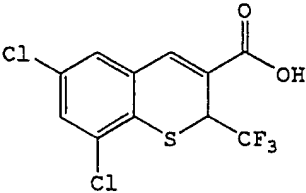
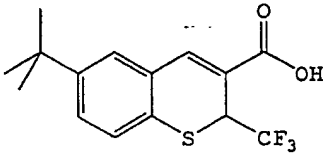
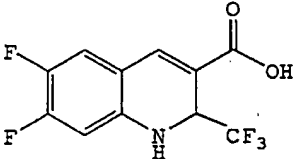
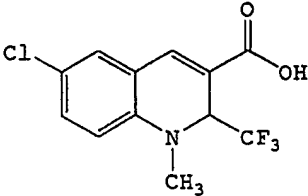
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5 Table 1. Examples of Chromene COX-2 Selective Inhibitors as Embodiments

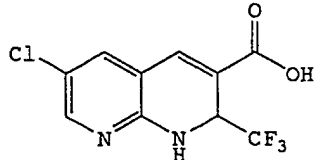
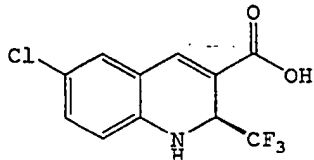
| <u>Compound Number</u> | <u>Structural Formula</u>   |
|------------------------|---|
| B-3                    |  <p>6-Nitro-2-trifluoromethyl-2H-1-benzopyran-3-carboxylic acid</p>                                 |
| B-4                    |  <p>6-Chloro-8-methyl-2-trifluoromethyl-2H-1-benzopyran-3-carboxylic acid</p>                     |
| B-5                    |  <p>((S)-6-Chloro-7-(1,1-dimethylethyl)-2-(trifluoromethyl)-2H-1-benzopyran-3-carboxylic acid</p> |

| <u>Compound Number</u> | <u>Structural Formula</u>   |
|------------------------|---|
| B-6                    |  <p>2-Trifluoromethyl-2H-naphtho[2,3-b]pyran-3-carboxylic acid</p>                        |
| B-7                    |  <p>6-Chloro-7-(4-nitrophenoxy)-2-(trifluoromethyl)-2H-1-benzopyran-3-carboxylic acid</p> |
| B-8                    |  <p>((S)-6,8-Dichloro-2-(trifluoromethyl)-2H-1-benzopyran-3-carboxylic acid</p>         |

| <u>Compound<br/>Number</u> | <u>Structural Formula</u>   |
|----------------------------|---|
| B-9                        |  <p>6-Chloro-2-(trifluoromethyl)-4-phenyl-2H-1-benzopyran-3-carboxylic acid</p>               |
| B-10                       |  <p>6-(4-Hydroxybenzoyl)-2-(trifluoromethyl)-2H-1-benzopyran-3-carboxylic acid</p>           |
| B-11                       |  <p>2-(Trifluoromethyl)-6-[(trifluoromethyl)thio]-2H-1-benzothiopyran-3-carboxylic acid</p> |

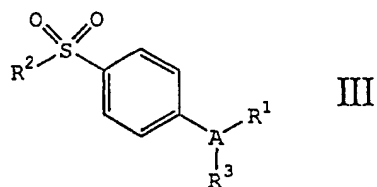
| <u>Compound Number</u> | <u>Structural Formula</u>   |
|------------------------|---|
| B-12                   |  <p>6,8-Dichloro-2-(trifluoromethyl)-2H-1-benzothiopyran-3-carboxylic acid</p>          |
| B-13                   |  <p>6-(1,1-Dimethylethyl)-2-(trifluoromethyl)-2H-1-benzothiopyran-3-carboxylic acid</p> |
| B-14                   |  <p>6,7-Difluoro-1,2-dihydro-2-(trifluoromethyl)-3-quinolinecarboxylic acid</p>       |
| B-15                   |  <p>6-Chloro-1,2-dihydro-1-methyl-2-(trifluoromethyl)-3-quinolinecarboxylic acid</p>  |



| <u>Compound Number</u> | <u>Structural Formula</u>   |
|------------------------|---|
| B-16                   |  <p>6-Chloro-2-(trifluoromethyl)-1,2-dihydro<br/>[1,8]naphthyridine-3-carboxylic acid</p> |
| B-17                   |  <p>((S)-6-Chloro-1,2-dihydro-2-(trifluoro<br/>methyl)-3-quinolinecarboxylic acid</p>     |

In a more preferred embodiment of the invention the cyclooxygenase-2 selective inhibitor is the substituted benzopyran (S)-6,8-dichloro-2-(trifluoromethyl)-2H-1-benzopyran-3-carboxylic acid, Formula B-8, or a pharmaceutically acceptable salt or derivative or prodrug thereof.

In a further preferred embodiment of the invention the cyclooxygenase inhibitor is selected from the class of tricyclic cyclooxygenase-2 selective inhibitors represented by the general structure of Formula III



wherein A is a substituent selected from partially unsaturated or unsaturated heterocyclyl and partially unsaturated or unsaturated carbocyclic rings;

wherein R<sup>1</sup> is at least one substituent selected from heterocyclyl, cycloalkyl, cycloalkenyl and aryl, wherein R<sup>1</sup> is optionally substituted at a substitutable position with one or more radicals selected from alkyl, haloalkyl, cyano, carboxyl, alkoxycarbonyl, hydroxyl, hydroxyalkyl, haloalkoxy, amino, alkylamino, arylamino, nitro, alkoxyalkyl, alkylsulfinyl, halo, alkoxy and alkylthio;

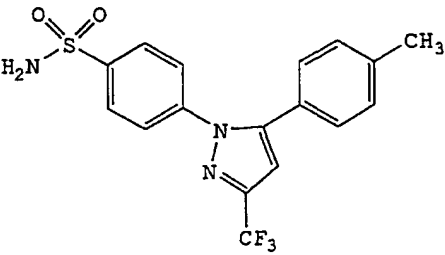
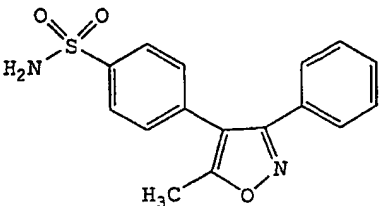
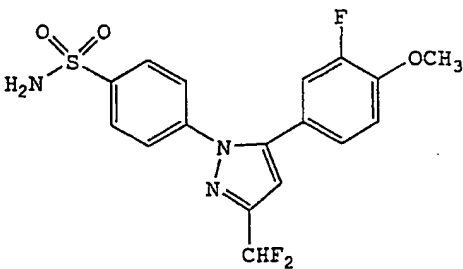
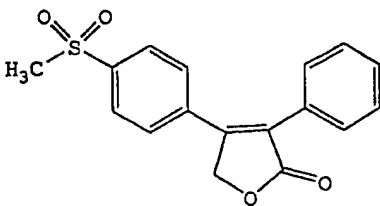
wherein R<sup>2</sup> is methyl or amino; and

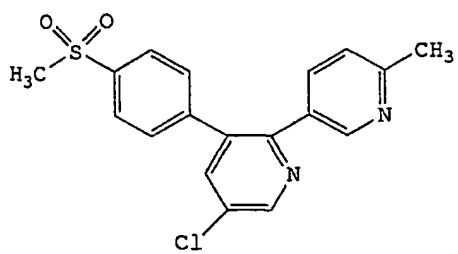
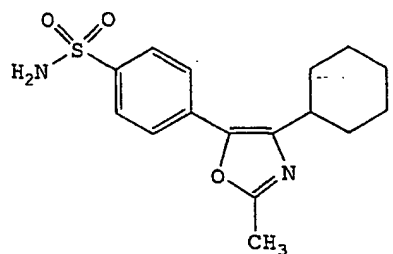
wherein R<sup>3</sup> is a radical selected from hydrido, halo, alkyl, alkenyl, alkynyl, oxo, cyano, carboxyl, cyanoalkyl, heterocyclyloxy, alkyloxy, alkylthio, alkylcarbonyl, cycloalkyl, aryl, haloalkyl, heterocyclyl, cycloalkenyl, aralkyl, heterocyclylalkyl, acyl, alkylthioalkyl, hydroxyalkyl, alkoxycarbonyl, arylcarbonyl, aralkylcarbonyl, aralkenyl, alkoxyalkyl, arylthioalkyl, aryloxyalkyl, aralkylthioalkyl, aralkoxyalkyl, alkoxyaralkoxyalkyl, alkoxycarbonylalkyl, aminocarbonyl, aminocarbonylalkyl, alkylaminocarbonyl, N-arylaminoalkyl, N-alkyl-N-arylaminoalkyl,

alkylaminocarbonylalkyl, carboxyalkyl, alkylamino,  
N-arylamino, N-aralkylamino, N-alkyl-N-  
aralkylamino, N-alkyl-N-arylamino, aminoalkyl,  
alkylaminoalkyl, N-arylaminoalkyl, N-  
5 aralkylaminoalkyl, N-alkyl-N-aralkylaminoalkyl, N-  
alkyl-N-arylaminoalkyl, aryloxy, aralkoxy,  
arylthio, aralkylthio, alkylsulfinyl,  
alkylsulfonyl, aminosulfonyl, alkylaminosulfonyl,  
N-arylaminosulfonyl, arylsulfonyl, N-alkyl-N-  
10 arylaminosulfonyl; or a pharmaceutically acceptable  
salt or derivative thereof.

In a still more preferred embodiment of the  
invention the cyclooxygenase-2 selective inhibitor  
represented by the above Formula III is selected from  
15 the group of compounds, illustrated in Table 2,  
consisting of celecoxib (B-18), valdecoxib (B-19),  
deracoxib (B-20), rofecoxib (B-21), etoricoxib (MK-663;  
B-22), JTE-522 (B-23), or a pharmaceutically acceptable  
salt or derivative or prodrug thereof.

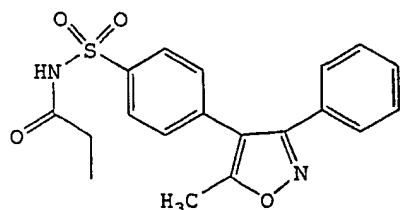
Table 2. Examples of Tricyclic COX-2 Selective  
Inhibitors as Embodiments

| <u>Compound<br/>Number</u> | <u>Structural Formula</u>  |
|----------------------------|--|
| B-18                       |    |
| B-19                       |   |
| B-20                       |  |
| B-21                       |  |

| <u>Compound Number</u> | <u>Structural Formula</u>   |
|------------------------|---|
| B-22                   | <br><chem>CS(=O)(=O)c1ccc(cc1)-c2cc(Cl)nc(C3=CC=CC=N3C)cc2</chem> |
| B-23                   | <br><chem>CS1=C(N2C=CC=C2S(=O)(=O)N)N=C1C3CCCCC3</chem>          |

In an even more preferred embodiment of the invention the COX-2 selective inhibitor is selected from the group consisting of celecoxib, rofecoxib and  
5 etoricoxib.

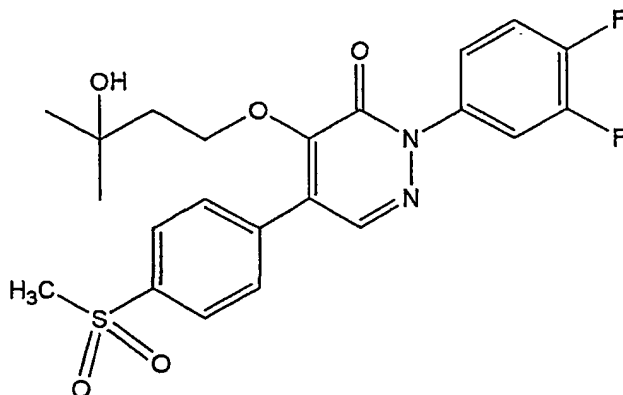
In another highly preferred embodiment of the invention parecoxib, B-24, which is a therapeutically effective prodrug of the tricyclic cyclooxygenase-2 selective inhibitor valdecoxib, B-19, may be  
10 advantageously employed as a source of a cyclooxygenase inhibitor (US 5,932,598, herein incorporated by reference).



B-24

In another preferred embodiment of the invention, the compound ABT-963 having the formula B-25 that has been previously described in International Publication number WO 00/24719, is another tricyclic cyclooxygenase-2 selective inhibitor which may be advantageously employed.

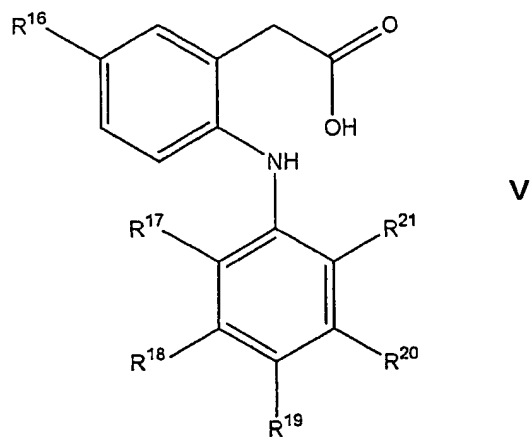
10



B-25

In a further preferred embodiment of the invention the cyclooxygenase inhibitor can be selected from the class of phenylacetic acid derivative cyclooxygenase-2 selective inhibitors represented by the general structure of Formula V:

15



wherein  $R^{16}$  is methyl or ethyl;

$R^{17}$  is chloro or fluoro;

5  $R^{18}$  is hydrogen or fluoro

$R^{19}$  is hydrogen, fluoro, chloro, methyl, ethyl, methoxy, ethoxy or hydroxy;

$R^{20}$  is hydrogen or fluoro; and

$R^{21}$  is chloro, fluoro, trifluoromethyl or methyl,

10 provided that  $R^{17}$ ,  $R^{18}$ ,  $R^{19}$  and  $R^{20}$  are not all fluoro when  $R^{16}$  is ethyl and  $R^{19}$  is H.

A particularly preferred phenylacetic acid derivative cyclooxygenase-2 selective inhibitor that is described in WO 99/11605 is a compound that has the designation of COX189 (CAS RN 346670-74-4), and that has the structure shown in Formula V,

wherein  $R^{16}$  is ethyl;

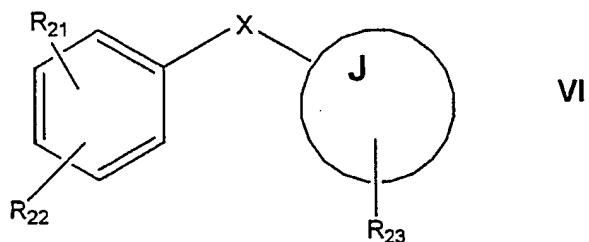
$R^{17}$  and  $R^{19}$  are chloro;

$R^{18}$  and  $R^{20}$  are hydrogen; and

20 and  $R^{21}$  is methyl.

Other preferred cyclooxygenase-2 selective inhibitors that can be used in the present invention

have the general structure shown in formula VI, where the J group is a carbocycle or a heterocycle. Particularly preferred embodiments have the structure:



5

where:

X is O; J is 1-phenyl; R<sub>21</sub> is 2-NHSO<sub>2</sub>CH<sub>3</sub>; R<sub>22</sub> is 4-NO<sub>2</sub>; and there is no R<sub>23</sub> group, (nimesulide), and

10 X is O; J is 1-oxo-inden-5-yl; R<sub>21</sub> is 2-F; R<sub>22</sub> is 4-F; and R<sub>23</sub> is 6-NHSO<sub>2</sub>CH<sub>3</sub>, (flosulide); and

X is O; J is cyclohexyl; R<sub>21</sub> is 2-NHSO<sub>2</sub>CH<sub>3</sub>; R<sub>22</sub> is 5-NO<sub>2</sub>; and there is no R<sub>23</sub> group, (NS-398); and

X is S; J is 1-oxo-inden-5-yl; R<sub>21</sub> is 2-F; R<sub>22</sub> is 4-F; and R<sub>23</sub> is 6-N<sup>-</sup>SO<sub>2</sub>CH<sub>3</sub> · Na<sup>+</sup>, (L-745337); and

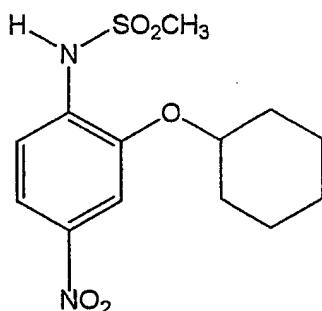
15 X is S; J is thiophen-2-yl; R<sub>21</sub> is 4-F; there is no R<sub>22</sub> group; and R<sub>23</sub> is 5-NHSO<sub>2</sub>CH<sub>3</sub>, (RWJ-63556); and

X is O; J is 2-oxo-5(R)-methyl-5-(2,2,2-trifluoroethyl)furan-(5H)-3-yl; R<sub>21</sub> is 3-F; R<sub>22</sub> is 4-F; and R<sub>23</sub> is 4-(p-SO<sub>2</sub>CH<sub>3</sub>)C<sub>6</sub>H<sub>4</sub>, (L-784512).

20 Further information on the applications of N-(2-cyclohexyloxynitrophenyl)methane sulfonamide (NS-398, CAS RN 123653-11-2), having a structure as shown in formula B-26, have been described by, for example, Yoshimi, N. et al., in

25





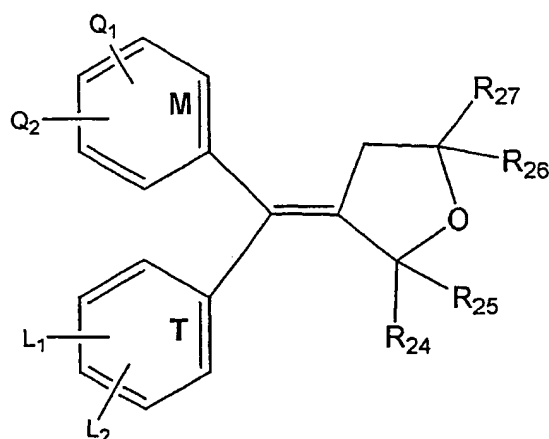
B-26

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Japanese *J. Cancer Res.*, 90(4):406 - 412 (1999);  
Falgueyret, J.-P. et al., in *Science Spectra*, available  
at: [http://www.gbhap.com/Science\\_Spectra/20-1-](http://www.gbhap.com/Science_Spectra/20-1-article.htm)  
article.htm (06/06/2001); and Iwata, K. et al., in *Jpn.*  
10 *J. Pharmacol.*, 75(2):191 - 194 (1997).

An evaluation of the antiinflammatory activity of  
the cyclooxygenase-2 selective inhibitor, RWJ 63556, in  
a canine model of inflammation, was described by  
Kirchner et al., in *J Pharmacol Exp Ther* 282, 1094-1101  
15 (1997).

Other compounds useful as the cyclooxygenase-2  
selective inhibitor in the present invention include  
diarylmethylidenefuran derivatives such as those  
described in U.S. Patent No. 6,180,651. Such  
20 diarylmethylidenefuran derivatives have the general  
formula shown below in formula VII:



VII

wherein:

the rings T and M independently are:

a phenyl radical,

5 a naphthyl radical,

a radical derived from a heterocycle comprising 5 to 6 members and possessing from 1 to 4 heteroatoms, or

a radical derived from a saturated hydrocarbon ring having from 3 to 7 carbon atoms;

10 at least one of the substituents Q<sub>1</sub>, Q<sub>2</sub>, L<sub>1</sub> or L<sub>2</sub> is:

an --S(O)<sub>n</sub> --R group, in which n is an integer equal to 0, 1 or 2 and R is a

lower alkyl radical having 1 to 6 carbon atoms or

a lower haloalkyl radical

15 having 1 to 6 carbon atoms, or

an --SO<sub>2</sub>NH<sub>2</sub> group;

and is located in the para position,

the others independently being:

a hydrogen atom,

20 a halogen atom,

a lower alkyl radical having 1 to 6 carbon atoms,

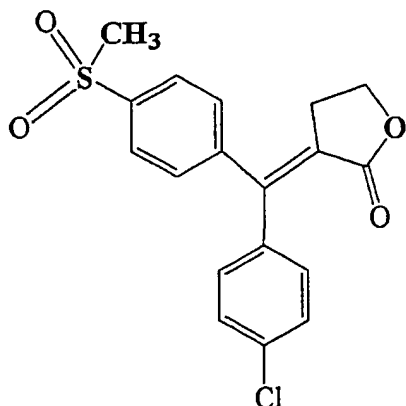
- a trifluoromethyl radical, or  
a lower O-alkyl radical having 1 to 6 carbon atoms,  
or  
Q<sub>1</sub> and Q<sub>2</sub> or L<sub>1</sub> and L<sub>2</sub> are a methylenedioxy group; and  
5 R<sub>24</sub>, R<sub>25</sub>, R<sub>26</sub> and R<sub>27</sub> independently are:  
a hydrogen atom,  
a halogen atom,  
a lower alkyl radical having 1 to 6 carbon atoms,  
a lower haloalkyl radical having 1 to 6 carbon  
10 atoms, or  
an aromatic radical selected from the group  
consisting of phenyl, naphthyl, thienyl, furyl  
and pyridyl; or,  
R<sub>24</sub>, R<sub>25</sub> or R<sub>26</sub>, R<sub>27</sub> are an oxygen atom, or  
15 R<sub>24</sub>, R<sub>25</sub> or R<sub>26</sub>, R<sub>27</sub>, together with the carbon atom to  
which they are attached, form a saturated  
hydrocarbon ring having from 3 to 7 carbon atoms;  
or an isomer or prodrug thereof.

Particular materials that are included in this  
20 family of compounds, and which can serve as the  
cyclooxygenase-2 selective inhibitor in the present  
invention, include N-(2-cyclohexyloxynitrophenyl)methane  
sulfonamide, and (E)-4-[(4-methylphenyl)(tetrahydro-2-  
oxo-3-furanylidene) methyl]  
25 benzenesulfonamide.

Preferred cyclooxygenase-2 selective inhibitors  
that are useful in the present invention include the  
following individual compounds; darbufelone (Pfizer),  
CS-502 (Sankyo), LAS 34475 (Almirall Profesfarma), LAS  
30 34555 (Almirall Profesfarma), S-33516 (Servier), SD 8381

(Pharmacia, described in U.S. Patent No. 6,034,256), BMS-347070 (Bristol Myers Squibb, described in U.S. Patent No. 6,180,651), MK-966 (Merck), L-783003 (Merck), T-614 (Toyama), D-1367 (Chiroscience), L-748731 (Merck), CT3 (Atlantic Pharmaceutical), CGP-28238 (Novartis), BF-389 (Biofor/Scherer), GR-253035 (Glaxo Wellcome), 6-dioxo-9H-purin-8-yl-cinnamic acid (Glaxo Wellcome), and S-2474 (Shionogi).

Another preferred embodiment of the invention, is  
10 the compound BMS-347070, having the formula:



C-69

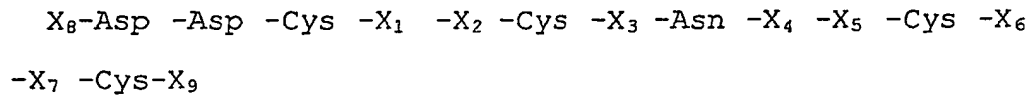
Information about S-33516, mentioned above, can be found in *Current Drugs Headline News*, at <http://www.current-drugs.com/NEWS/Inflam1.htm>, 10/04/2001, where it was reported that S-33516 is a tetrahydroisoinde derivative which has  $IC_{50}$  values of 0.1 and 0.001 mM against cyclooxygenase-1 and cyclooxygenase-2, respectively. In human whole blood, S-33516 was reported to have an  $ED_{50}$  = 0.39 mg/kg.

All references, patents or applications U.S. or foreign, cited in the application are hereby incorporated by reference as if written herein.

The explanations and illustrations presented herein  
5 are intended to acquaint others skilled in the art with  
the invention, its principles, and its practical  
application. Those skilled in the art may adapt and  
apply the invention in its numerous forms, as may be  
best suited to the requirements of a particular use.  
10 Accordingly, the specific embodiments of the present  
invention as set forth are not intended as being  
exhaustive or limiting of the invention.

## WHAT IS CLAIMED IS:

1. A method for the treatment or prevention of intestinal polyps in a subject, the method comprising administering to the subject an amount of a polypeptide  
5 and an amount of a cyclooxygenase-2 selective inhibitor wherein the amount of the polypeptide and the amount of the cyclooxygenase-2 selective inhibitor together comprise an intestinal polyp treating-effective amount of the polypeptide and the cyclooxygenase-2 selective  
10 inhibitor, wherein the polypeptide has the formula:



15 and wherein each of  $X_1$ ,  $X_2$ ,  $X_3$ ,  $X_4$ ,  $X_5$ ,  $X_6$ , and  $X_7$  is an amino acid residue,  $X_8$  and  $X_9$  are independently hydrogen or at least one amino acid residue, and

the polypeptide is cross-linked by a disulfide bond between the cystine residue immediately adjacent the  
20 amine group of  $X_1$  and the cystine residue immediately adjacent the amine group of  $X_6$  and by a disulfide bond between the cystine residue immediately adjacent the amine group of  $X_3$  and the cystine residue immediately adjacent the carboxy group of  $X_7$ .

2. A process of claim 1 wherein the polypeptide and cyclooxygenase-2 inhibitor are present as a single composition.

5        3. A process of claim 2 wherein the concentration of the peptide in the composition is at least 0.0001 percent by weight of the composition.

10       4. A process of claim 2 wherein the concentration of the peptide in the composition is at least 0.001 percent by weight of the composition.

15       5. A process of claim 2 wherein the concentration of the peptide in the composition is at least 0.01 percent by weight of the composition.

20       6. A process of claim 2 wherein the concentration of the peptide in the composition is at least 0.1 percent by weight of the composition.

7. A process of claim 2 wherein the concentration of the peptide in the composition is at least 1 percent by weight of the composition.

8. The process of claim 1 wherein said subject has been determined to have a genetic predisposition for the growth of polyps in the intestine.

5        9. The process of claim 1 wherein polyps have been identified in the intestine of said subject.

10       10. The process of claim 1 wherein said subject has been identified as having intestine cancer.

11. A process of claim 2 wherein the concentration of the peptide in the composition is at least 0.0001 percent by weight of the composition.

15       12. A process of claim 2 wherein the concentration of the peptide in the composition is at least 0.001 percent by weight of the composition.

20       13. A process of claim 2 wherein the concentration of the peptide in the composition is at least 0.01 percent by weight of the composition.

25       14. A process of claim 2 wherein the concentration of the peptide in the composition is at least 0.1 percent by weight of the composition.



15. A process of claim 2 wherein the concentration of the peptide in the composition is at least 1 percent by weight of the composition.

16. The process of claim 2 wherein said subject has been determined to have a genetic predisposition for the growth of polyps in the intestine.

5        17. The process of claim 2 wherein the polyps have been identified in the intestine of said subject.

18. The process of claim 2 wherein said subject has been identified as having intestine cancer.

10

19. The process of claim 1 wherein  $X_1$  is selected from the group of amino acid residues consisting of aspartic acid, glutamic acid, glycine, lysine, asparagine, proline, glutamine, arginine, serine, and  
15 threonine.

20. The process of claim 1 wherein  $X_1$  is selected from the group of amino acid residues consisting of glutamic acid, arginine, lysine, serine, aspartic acid,  
20 asparagine, glutamine, and glycine.

21. The process of claim 1 wherein  $X_1$  is selected from the group of amino acid residues consisting of glutamic acid, aspartic acid, arginine, and lysine.

25

22. The process of claim 1 wherein  $X_1$  is glutamic acid.

23. The process of claim 1 wherein  $X_2$  is selected  
5 from the group of amino acid residues consisting of  
leucine, isoleucine, tyrosine, phenylalanine,  
tryptophan, valine, methionine, cysteine, alanine,  
histidine, proline, threonine, glycine, asparagine, and  
glutamine.

10

24. The process of claim 1 wherein  $X_2$  is selected  
from the group of amino acid residues consisting of  
cysteine, phenylalanine, glycine, isoleucine, leucine,  
methionine, valine, and tyrosine.

15

25. The process of claim 1 wherein  $X_2$  is selected  
from the group of amino acid residues consisting of  
leucine, isoleucine, tyrosine, valine, methionine.

20

26. The process of claim 1 wherein  $X_2$  is selected  
from the group of amino acid residues consisting of  
leucine, and isoleucine.

27. The process of claim 1 wherein  $X_2$  is leucine.

25

28. The process of claim 1 wherein  $X_3$  is selected from the group of amino acid residues consisting of valine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, methionine, cysteine, alanine, histidine,  
5 proline, threonine, glycine, glutamine, asparagine, and serine.

29. The process of claim 1 wherein  $X_3$  is selected from the group of amino acid residues consisting of  
10 valine, isoleucine, leucine, tyrosine, phenylalanine, methionine, cysteine, alanine, histidine, and proline.

30. The process of claim 1 wherein  $X_3$  is selected from the group of amino acid residues consisting of  
15 valine, isoleucine, leucine, methionine, and cysteine.

31. The process of claim 1 wherein  $X_3$  is valine.

32. The process of claim 1 wherein  $X_3$  is  
20 isoleucine.

33. The process of claim 1 wherein  $X_4$  is selected from the group of amino acid residues consisting of valine, isoleucine, leucine, tyrosine,  
25 phenylalanine, tryptophan, methionine, cysteine,

alanine, histidine, proline, threonine, glycine, glutamine, asparagine, and serine.

34. The process of claim 1 wherein  $X_4$  is selected  
5 from the group of amino acid residues consisting of valine, isoleucine, leucine, tyrosine, phenylalanine, methionine, cysteine, alanine, histidine, and proline.

35. The process of claim 1 wherein  $X_4$  is selected  
10 from the group of amino acid residues consisting of valine, isoleucine, leucine, methionine, and cysteine.

36. The process of claim 1 wherein  $X_4$  is valine.

15 37. The process of claim 1 wherein  $X_5$  is alanine, histidine, cysteine, methionine, valine, leucine, isoleucine, tyrosine, phenylalanine, proline, threonine, glycine, glutamine, asparagine, and serine.

20 38. The process of claim 1 wherein  $X_5$  is selected from the group of amino acid residues consisting of alanine, histidine, cysteine, methionine, valine, proline, threonine, glycine, glutamine, asparagine, and serine.

25

39. The process of claim 1 wherein  $X_5$  is selected from the group of amino acid residues consisting of alanine, histidine, cysteine, proline, threonine, glycine, glutamine, asparagine, and serine.

5

40. The process of claim 1 wherein  $X_5$  is alanine.

41. The process of claim 1 wherein  $X_6$  is selected from the group of amino acid residues consisting of  
10 threonine, proline, alanine, histidine, cysteine, methionine, valine, leucine, isoleucine, tyrosine, glycine, glutamine, asparagine, and serine.

42. The process of claim 1 wherein  $X_6$  is selected  
15 from the group of amino acid residues consisting of threonine, proline, alanine, histidine, cysteine, methionine, glycine, glutamine, asparagine, and serine.

43. The process of claim 1 wherein  $X_6$  is selected  
20 from the group of amino acid residues consisting of threonine, proline, alanine, histidine, and glycine.

44. The process of claim 1 wherein  $X_6$  is threonine.

25

45. The process of claim 1 wherein X<sub>7</sub> is selected from the group of amino acid residues consisting of glycine, threonine, proline, alanine, histidine, cysteine, methionine, valine, leucine, isoleucine, 5 glutamine, asparagine, serine, glutamic acid, and aspartic acid.

46. The process of claim 1 wherein X<sub>7</sub> is selected from the group of amino acid residues consisting of 10 glycine, threonine, proline, alanine, histidine, cysteine, glutamine, asparagine, and serine.

47. The process of claim 1 wherein X<sub>7</sub> is selected from the group of amino acid residues consisting of 15 glycine, threonine, proline, alanine, histidine, glutamine, asparagine, and serine.

48. The process of claim 1 wherein X<sub>7</sub> is glycine.

20 49. The process of claim 1 wherein the polypeptide is uroguanylin.

50. The process of claim 1 wherein the polypeptide is human uroguanylin.

51. The process of claim 1 wherein the composition comprises pro-uroguanylin.

52. The process of claim 1 wherein the composition  
5 comprises human pro-uroguanylin.

53. The process of claim 1 wherein the composition comprises guanylin.

10 54. The process of claim 1 wherein the composition comprises lymphoguanylin.

55. The process of claim 1 wherein the composition comprises prolymphoguanylin.

15

56. The process of claim 1 wherein the composition comprises heat stable enterotoxin.

57. The process of claim 1 wherein the composition  
20 comprises a polypeptide, which is degraded with endogenous proteases of the subject, into uroguanylin.

58. The process of claim 1 wherein about 0.5 mg to about 2 mg of the polypeptide is administered per  
25 kilogram of the subjects weight.



59. The process of claim 1 wherein the subject is human.

5        60. The process of claim 1 wherein said peptides are administered in a pharmaceutical composition which contains said peptide and one or more pharmacologically acceptable, inert or physiologically active diluents of adjuvants.

10

61. The process of claim 1 wherein  $X_1$  is glutamic acid,  $X_2$  is leucine,  $X_3$  is isoleucine,  $X_4$  is valine,  $X_5$  is alanine,  $X_6$  is threonine, and  $X_7$  is glycine.

15        62. A process for the prevention, inhibition and treatment of cancer in the intestine of a subject, the process comprising administering to the subject the composition of claim 1.

20        63. A process for the prevention, inhibition and treatment of cancer in the intestine of a subject, the process comprising administering to the subject the composition of claim 2.

64. The process of claim 62 wherein the composition comprises uroguanylin.

65. The process of claim 63 wherein the  
5 composition comprises uroguanylin.

66. The process of claim 62 wherein the composition comprises pro-uroguanylin.

10 67. The process of claim 63 wherein the composition comprises pro-uroguanylin.

68. A process for retarding the development of polyps and prevention, inhibition and treatment of  
15 polyps in the intestine of a subject, the process comprising administering to the subject a composition comprising an agonist peptide or compound which binds to a guanylate cyclase receptor GC-C in the intestine of the subject, in combination with a cyclooxygenase-2  
20 inhibitor.

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
15 August 2002 (15.08.2002)

PCT

(10) International Publication Number  
**WO 02/062369 A3**

(51) International Patent Classification<sup>7</sup>: **A61K 31/00**,  
38/03, 38/10 // (A61K 38/03, 31:00) (A61K 38/10, 31:00)

(21) International Application Number: PCT/US02/03201

(22) International Filing Date: 4 February 2002 (04.02.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/265,955 2 February 2001 (02.02.2001) US

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

(88) Date of publication of the international search report:  
28 August 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **UROGUANYLIN AND CYCLOOXYGENASE-2 INHIBITOR COMBINATIONS FOR INHIBITION OF INTESTINAL CANCER**

(57) Abstract: Disclosed is a method of retarding the development of polyps and prevention, inhibition and treatment of cancer in the intestine of a subject by administration of a composition comprising a peptide with the active domain of uroguanylin or any agonist peptide or compound binding to the guanylate cyclase receptor GC-C in the intestine in combination with a naturally occurring, derived from a naturally occurring, or a chemically synthesized cyclooxygenase-2 inhibitor, preferably a selective cyclooxygenase-2 inhibitor.



**WO 02/062369 A3**

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 02/03201

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/00 A61K38/03 A61K38/10 //(A61K38/03,31:00),  
(A61K38/10,31:00)

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, MEDLINE, EMBASE, BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|------------|---|-----------------------|
| Y          | <p>WO 00 23433 A (DEVADAS BALEKUDRU ;GRANETO<br/>MATTHEW J (US); BROWN DAVID L (US); SEA)<br/>27 April 2000 (2000-04-27)<br/>page 3, line 1 - line 3<br/>page 12, line 16 -page 13, line 15<br/>---<br/>-/-</p> | 1-68                  |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

\*A\* document defining the general state of the art which is not considered to be of particular relevance

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\*P\* document published prior to the international filing date but later than the priority date claimed

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\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search

19 May 2003

Date of mailing of the international search report

26/05/2003

Name and mailing address of the ISA

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 02/03201

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|------------|---|-----------------------|
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| A          | WO 98 47890 A (DEVADAS BALEKUDRU ;GRANETO MATTHEW J (US); BROWN DAVID L (US); SEA)<br>29 October 1998 (1998-10-29)<br>page 3, line 24 -page 6, line 8<br>page 8, line 8 -page 9, line 23  | 1-68                  |
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| A          | FORTE L R: "GUANYLIN REGULATORY PEPTIDES: STRUCTURES, BIOLOGICAL ACTIVITIES MEDIATED BY CYCLIC GMP AND PATHOBIOLOGY"<br>REGULATORY PEPTIDES, ELSEVIER SCIENCE BV, NL,<br>vol. 81, no. 1 - 3,<br>31 May 1999 (1999-05-31), pages 25-39,<br>XP000979549<br>ISSN: 0167-0115<br>abstract<br>page 26, left-hand column -right-hand column<br>page 27, left-hand column, line 3 - line 10<br>page 36, left-hand column, paragraph 2<br>figures 1,3              | 1-68                  |

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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 02/03201

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: —  
because they relate to subject matter not required to be searched by this Authority, namely:  
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; It is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1-68 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

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Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 02/03201

| Patent document<br>cited in search report |               | Publication<br>date |            | Patent family<br>member(s) |  | Publication<br>date |
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